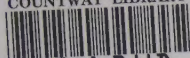
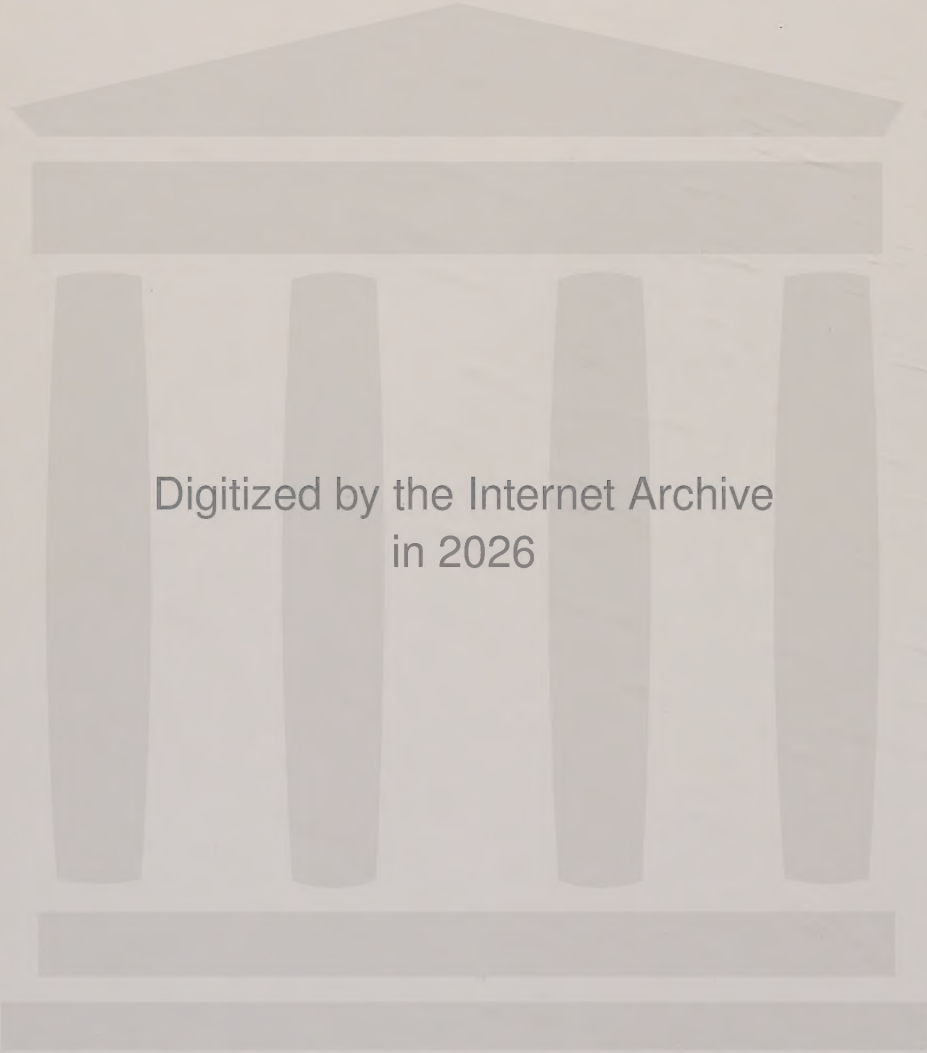


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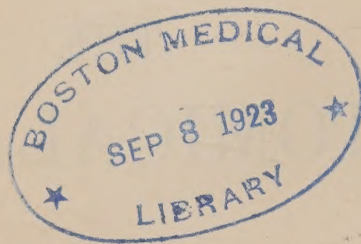
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Resumen por el autor, I. E. Wallin.

Sobre la naturaleza de las mitocondrias

V. Un análisis crítico de la obra de Portier "Les
symbiotes"

Portier en 1918 ha llegado a una concepción bacterial de las mitocondrias. Dicho autor creó que los "symbiotes" (mitocondrias) entran y salen del organismo constantemente. Los "symbiotes" son organismos omnipresentes que exhiben extrema resistencia a los agentes físicos y químicos.

Translation by José F. Nonidez
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ON THE NATURE OF MITOCHONDRIA

V. A CRITICAL ANALYSIS OF PORTIER'S 'LES SYMBIOTES'

IVAN E. WALLIN

TRANSLATION BY LOUIS LABARRERE

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Since the publication of "Die Elementarorganismen und ihre Beziehungen zu den Zellen" in 1890 there have been only a few expressions of opinion which have leaned towards Altmann's conception of mitochondria. These expressions have been more of the nature of opinions based on generalities than convictions arrived at from experimental and observational evidence. The major part of mitochondrial literature, particularly that of recent years, has attempted to establish a cytoplasmic origin for mitochondria. The success of this adventure may be measured by the uncertainty and indifference with which this conception has been received by biologists in general. It appears that the basis for a conception of cytoplasmic origin of these structures rests chiefly on topographical relationship. In its final analysis such a basis carries no more proof of origin than may be found in the old adage: "possession is nine points of the law."

Portier's 'Les Symbiotes' ('18) is, apparently the first pretentious experimental work which supports, in part, Altmann's conception of mitochondria. While Portier's opinion differs from Altmann's conceptions in many particulars, the two opinions agree in the essential part of the hypothesis, namely, that mitochondria are organisms of a bacterial nature. Independent of Portier's researches and on the basis of experimental grounds and evidence by analogy, the writer (Wallin 1922 a, b) has also arrived at a bacterial conception of mitochondria. The writer's conceptions were arrived at from different lines of investigation than those employed by Portier. His conceptions are also

quite different from Portier's, especially in regard to the origin of mitochondria.

Portier's hypothesis may be better stated by quoting from the preface of 'Les Symbiotes': "*Chaque cellule vivante renferme dans son protoplasme des formations que les histologistes designent sous le nom de mitochondries. Ces organites ne seraient pour moi autre chose que des bacteries symbiotiques, ce que je nomme des 'symbiotes' . . . La bacterie symbiotique vient du milieu exterieur: elle peut, dans certains cas, y retourner et vivre d'une vie independante. Les bacteries seraient donc les seuls etres simples; tous les autres seraient doubles.*"

The chief basis for Portier's conclusions rests on his results from attempts to grow mitochondria in artificial culture media. The results in these attempts have not been successful with all tissues. The successful growths were obtained chiefly from the fat in relation to the sex organs, particularly the testes. Attempts at cultivating the mitochondria of the pancreas and liver, for example, have been unsuccessful. This failure is accounted for on the basis of his general theory regarding the nature and relationship of mitochondria. Portier believes that the 'symbiotes' are especial microorganisms found in great abundance in nature. They are constantly entering and leaving the host organism under normal conditions. After entering a host they first occupy an intercellular position and later take up an intracellular relationship. In the intercellular relationship the organisms retain their hardy properties and are easily cultivated in culture media. After the 'symbiotes' have taken up the intracellular position they lose their hardy qualities, develop fragility and are quite incapable of cultivation in culture media.

A remarkable characteristic of Portier's 'symbiotes' is their extraordinary resistance to chemical and physical agents. The organisms after a number of passages in culture may be brought to a moist temperature of 115° C. before they are destroyed. In a state of desiccation they resist temperatures up to 150° C. 'Symbiotes' in culture media resist the action of absolute alcohol and chloroform for a period of a year. Further, the organisms resist 5 per cent phenol for more than fifty hours, 20 per cent

formaldehyde for twenty five hours, and 10 per cent tincture of iodine for twenty four hours. The organisms may be exposed to the action of X-rays for considerable periods of time without being destroyed.

The temperature at which the 'symbiotes' were cultivated varied with the source of the 'symbiotes.' The organisms from higher vertebrates were best grown at a temperature of 40° C. while those from invertebrates were cultivated at a temperature of 25° C. In all the cultural experiments only one type of organism was produced for each temperature group. The culture media used were ordinary bacteriological media. The experiments were apparently conducted with particular precautions to guard against contaminations. The room in which the work was done was thoroughly scrubbed, the instruments used were sterilized in a flame and other general precautions were observed. The cultural experiments were repeated a number of times with constant results.

There is a great temptation to drift into fields of pure speculation when one is dealing with a problem of the nature of mitochondria. In a bacterial conception of these structures one faces a myriad of possibilities in life processes and biological relationships that suggest an entirely new and unique conception of life. Altmann could not resist this temptation and his speculations led him into conflict with established facts in biology. Portier discusses various functional relationships of 'symbiotes' to the cell, both normal and pathological. While these speculations are interesting their significance is dependent upon the determination of the nature of mitochondria.

The premises for Portier's researches rest on a statement attributed to Louis Pasteur in which he maintained that the tissues of a healthy animal are free from bacteria. This statement leads Portier to differentiate between the 'interior medium' which is aseptic and the 'exterior medium' which contains numerous microorganisms. It is difficult to determine just what Portier means by 'interior' and 'exterior' media. From the content of his dissertation it appears that the 'interior' medium is an intercellular medium and the 'exterior' medium

refers to any position that is physiologically outside of the body. Pasteur's statement concerning the aseptic condition of the tissues of healthy animals is undoubtedly erroneous. Medical literature in recent years contains a number of observations which refute this statement. Gurney-Dixon ('20) claims to have found bacteria present in 80 per cent of apparently healthy animals. The writer's experience in this direction does not agree with Pasteur's contention. In a few attempts I have been able to get luxuriant bacterial growth on the ordinary agar culture media from lymph nodes of apparently healthy young rabbits and kittens. While an attempt has not yet been made to determine the relative prevalence of bacteria in the tissues of apparently healthy animals, these preliminary experiments point to a relatively high percentage of prevalence.

If bacteria are normally present in the tissues of apparently healthy animals, it is obvious that the fundamental basis for Portier's experiments is faulty. Portier made his cultures from the tissues of adult animals. It is quite impossible to determine whether he was dealing with intercellular symbiotic organisms which are, apparently, normally present or if he actually produced cultures from mitochondria.

It is difficult to harmonize Portier's 'symbiotes' with the known microorganisms. Their remarkable resistance to physical and chemical agents would suggest a hitherto unknown type of organism. Obviously, with such characteristics they are not identical with mitochondria. That they are the extracellular representatives of mitochondria remains to be demonstrated. Such free living and hardy forms fit well into Portier's hypothesis. However, there are certain conditions which Portier has not taken into consideration, conditions which have a decided bearing on the solution of the origin of mitochondria. Portier believes that the 'symbiotes' are constantly entering the host organism by way of the alimentary tract. This assumption is based on various observations on the presence of bacteria in the intestinal lumen and cells of insects and rabbits. In the rabbit he found bacteria in the intestinal contents, and apparently the same strain of bacteria within the epithelial cells of the intestine. The

writer has made the same observation on a one-day old kitten. Portier further observed that some of the bacteria in the insect intestinal epithelium were digested within the cell. Some of the apparently ingested bacteria, however, he maintains are retained intact within the cell. There is very little room for doubt concerning the ingestion of some bacteria by the intestinal epithelial cells. However, there appears to be no relationship between this phenomenon and the origin of mitochondria. While it is possible that the intestinal tract may be the avenue of entry for 'new symbiotic complexes,' the perpetuation of such a new symbiotic complex is by way of the germ cell. The presence of mitochondria in the germ cell has been demonstrated beyond a doubt. Their increase by simple division in embryonic differentiation has also been observed. The possibility of a functional and morphological differentiation in the developing host is supported by the analogous conditions which are present in the differentiation of the symbionts in the higher lichens.

Aside from his results in the cultural experiments in 'symbiotes,' Portier has brought forth a number of observations which support a bacterial conception of mitochondria. A great portion of this evidence deals with the relationship of bacteria to insect life. These bacterial forms are not mitochondria. If they are 'symbiotes' they strengthen Portier's hypothesis, but this remains to be determined. However, in many instances they do furnish examples of bacterial relationships to higher organisms which are similar to the relationships of mitochondria to the cell.

It is quite certain that a definite transplantation of mitochondria to an artificial culture medium would constitute conclusive evidence of the organismal character of mitochondria. The failure to accomplish such growth, however, is not evidence of a cytoplasmic origin of mitochondria.

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Resumen por los autores, H. E. Jordan y F. Helvestine.

Ciliogénesis en el epidídimo de la rata blanca.

Esta investigación representa un esfuerzo para determinar la verdadera naturaleza de las pestañas vibrátiles y sus corpúsculos basales en las células que tapizan el conducto del epidídimo de la rata blanca. Comprende un estudio comparativo de la ciliogénesis en los conductos eferentes y el epidídimo propiamente dicho. Las pestañas de las células de los conductillos eferentes (conos vasculares) se producen mediante crecimiento de corpúsculos bacilares basales conspicuos, los cuales derivan del centrosoma. Las pestañas de las células que tapizan el conducto propiamente dicho representan porciones distales persistentes de la membrana celular y, en parte, restos espongioplásmicos del citoplasma, el cual ha desaparecido en su mayor parte distalmente durante la actividad secretora. Los gránulos basales representan engrosamientos nodales en el punto donde los filamentos espongioplásmicos pasan en el citoplasma no alterado y, en parte, bandas de cierre en las cuales permanecen insertas las porciones persistentes distales de la membrana.

Translation by José F. Nonidez
Cornell Medical College, New York,

CILIOGENESIS IN THE EPIDIDYMIS OF THE WHITE RAT

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ONE PLATE (SIX FIGURES)

INTRODUCTION

In a paper on 'Amitosis in the epididymis of the mouse,' the senior author (1913) described basal granules in connection with the cilia of the cells lining both the ductuli efferentes and the duct proper. The observation that the ciliated cells of the ductus epididymidis contain basal bodies has recently been sharply questioned by Reichel (1921). Reichel's study of the epididymis of the mole leads him to support the conclusion of Aigner (1900) and of Fuchs (1902) that the ciliated cells of the ductus epididymidis of mammals lack basal bodies. These discrepant observations focus interest anew upon the question of the genetic difference between motile and non-motile cilia.

The questions at issue are these: 1, Are the cilia of the cells lining the ductuli efferentes motile, and those lining the epididymis proper non-motile? 2, Are the former associated with basal granules, and do the latter lack such granules? 3, Assuming that the ciliated cells of the duct of the epididymis lack basal bodies in their definitive condition, is this the persistence of a primary condition or is it the result of a secondary modification?

The third question is suggested by the theoretic possibility that non-motile cilia might develop like motile cilia in relation to basal bodies, but that the basal bodies might secondarily disintegrate coincident with loss of ciliary motility. The cause of loss of motility on the part of the cilia might be the loss of integrity on the part of the basal bodies. The latter might follow the assumption of a predominant secretory function on the part of the ciliated cells.

The above outlined considerations are related to the hypothesis of Henneguy and von Lenhossek (1898) that the basal bodies are derivatives of the centrosome. Jordan (1913) supports this hypothesis and formulates the corollary that since ciliated cells have lost their centrosome they must of necessity divide by the amitotic mode. The truth of this corollary he has established by the observations of exclusively amitotic division on the part of ciliated cells, more especially those of the ductuli efferentes of the mouse. This conclusion of exclusive amitosis of ciliated cells has been confirmed by Saguchi (1917) for vertebrates and by Helvestine (1921) for invertebrates.

MATERIAL AND METHODS

The material investigated includes the testes and associated epididymides of four young white rats. These rats were respectively 20, 30, 39 and 41 days old. The three older specimens were furnished by The Wistar Institute. One testicle of each individual was fixed in strong Flemming's solution, the other in Helly's Zenker-formol mixture. Sections were cut at 5 microns and stained with iron-hematoxylin, with and without eosin counterstain.

Rats of the ages specified were employed on the assumption that ciliogenesis in the epididymis is closely related to the initial spermatogenic activity of the testis. The rat comes to sexual maturity at the end of the second month (Donaldson). It was assumed, therefore, that some time during the second month ciliogenesis took place in the epididymis. At 30 days the ductules are already lined with numerous ciliated cells. All stages in ciliogenesis also occur. This is true also in the 39- and 41-day-old specimens. In the 20-day specimen the ductus epididymidis is lined with cells lacking cilia. In the older specimens these cells are practically all ciliated, occasional cells showing early stages in ciliogenesis. In the 41-day specimen the predominant cell of the testis is the primary spermatocyte. Many spermatogonia also occur, and considerable numbers of secondary spermatocytes, and an occasional spermatid.

DUCTULI EFFERENTES

The following description applies specifically to the con vasculosi of the 41-day rat, but in general also to those of the 30- and 39-day specimens. The epithelium is generally two layered. The cell boundaries of the basal layer are not distinct, but appearances in certain more favorable areas indicate that the superficial cells send constricted ends among them to the basement membrane. The 'two layered' epithelium is therefore in general pseudostratified.

The majority of the ductuli as seen in transverse sections have all of the cells ciliated. The non-ciliated cells include the young undifferentiated elements and the older secreting cells. The ciliated cells carry cilia of differing lengths. Many carry cilia of a length approximately two-thirds the length of the cells. Large numbers of others have cilia approximately half the length of the longest cilia. These shorter cilia appear more densely matted. Other cells have mixed cilia of both lengths. Certain tubules as seen in cross section contain only cells of the type with long cilia. The majority of the tubules contain cells with the three dimensional varieties of cilia, including occasionally non-ciliated cells. The cells with the longest cilia contain a more distally located nucleus, which stains relatively more intensely, and a homogeneous more deeply-staining cytoplasm. The cytoplasm of the cells with the shorter cilia is extensively vacuolated and granulated.

Terminal bars are discernible in relation to all of the cells. The longest cilia are attached to conspicuous bacillary basal corpuscles. On closer examination some of these rod-like corpuscles are seen to have a dumb-bell-shaped form (figs. 2 and 3). In the cells with the shorter cilia the basal corpuscles are much less distinct. They can, however, be clearly recognized. They appear as smaller, spheroidal or granular, only lightly staining elements (figs. 2, 3, and 4). Occasionally mitotic figures occur in both the basal and the superficial cells. But the superficial cells, when in mitosis, are without cilia. They are for the most part in the preciliated stage. Judging from the character of the cytoplasm, however, a certain small number of

these dividing cells may be such as have previously lost their cilia. There are no indications of amitosis, so frequent at adult stages, in these cells.

The earliest stages in ciliogenesis are illustrated in figure 1. The distal portion of the cells contains an aggregation of small spherical, deeply-staining, granules. These migrate towards the border, ultimately in two tiers, the outermost becoming rod-shaped and sprouting cilia. It seems probable that the later dumb-bell-shaped appearance of the basal corpuscles may result from a more or less close fusion of these two tiers of granules.

This material gives no clear evidence of rootlets connected with the basal bodies. However, the somewhat striated appearance of the distal half of the cells (fig. 3) suggests the occurrence of rootlets. The bacillary basal corpuscles elongate somewhat (fig. 2) and the cilia grow to great lengths. Subsequently they apparently break about midway, become more or less closely clumped and matted, and the basal bodies suffer a coincident decrease of size (possibly a fragmentation) with loss of staining capacity. There are as yet no pockets of clearer non-ciliated cells, suggestive of glands, frequently described for the adult ductules. There is little direct indication of secretory activity at this stage. Only very occasionally is a cell encountered with deeply-staining cytoplasmic globules. Free globules along the luminal border are not discernible. However, the presence of a deeply-staining, finely granular coagulum within the lumens of the ductules, and the vacuolated character of the cytoplasm suggest some secretory activity. The cells with the short, matted cilia may be such as are entering upon the first phase of initial secretion.

The Flemming-fixed tissue gives no indication of mitochondria. Contrary to expectation, certain portions of the Helly-fixed tissue show the mitochondria conspicuously (fig. 5). In these regions the basal bodies are not preserved. Of considerable importance is the fact that the cytoplasm of the distal pole of these cells is free of mitochondria. This would seem to show conclusively that mitochondria are not directly concerned with the development of cilia. Cilia do not sprout from mitochondria as Saguchi supposed.

DUCTUS EPIDIDYIMIDIS

The ductus epididymidis of the 41-day-old rat is likewise lined with a stratified columnar, apparently ciliated, epithelium (fig. 6). The cilia here also, as in the ductuli efferentes, are apparently longer and shorter filaments. In the ductus, however, the cilia are uniformly matted. The terminal bars are conspicuous, but the deeply-stained bacillary sort of basal corpuscle is lacking. Certain cells here also have a homogeneous cytoplasm and a more deeply staining nucleus, but their basal corpuscles are only slightly more conspicuous than in the predominating cells with vacuolated and granular cytoplasm. Both mitotic spindles and amitotic nuclear divisions are relatively numerous. Other than the presence of occasional cells with large deeply-stained globules, and an occasional similar globule within the lumen, there is no distinct evidence of secretory activity. Spermatocytes, both primary and secondary, occasionally appear in the lumen.

When one compares a section of the duct of the epididymis of the older specimen with one of a ductulus efferens at a later stage of ciliogenesis, one gets the impression of a close similarity (compare figs. 4 and 6). One is tempted to draw the inference that the cells of the epididymis, with matted cilia and pale minute basal corpuscles, developed in the same way as the cells of similar appearance in the efferent ductules, that is, from cells with deeply staining bacillary basal corpuscles and distinct vibratile cilia. When one searches through material of earlier stages, however, one meets a clear negative to such inference. In the epididymis no cells can be found with conspicuous bacillary basal corpuscles like those of the efferent ductules. On the contrary, the basal corpuscles of the shorter cilia of the cells of the 30-day stage are even more difficult to discern than those of later stages. In sections similarly fixed and stained, from a still younger stage (approximately 20 days), the cells are cuboidal, and lack cilia and basal bodies altogether. Certain cells from the 30-day stage give the clue for the probably correct interpretation of these non-vibratile cilia of the ductus epididymidis. In brief, these cells show that these so-called cilia are not really

cilia in any proper sense at all, but more of the nature of a very wide and partially collapsed striated or brush border (cell at right in fig. 6). The younger cells show a striated border very similar to the columnar cells of the small intestine. The striations are more distinct and give more the impression of short robust cilia. As the cells grow in length, the striated border (ciliated border) likewise lengthens, resembling now somewhat the brush border of the convoluted tubules of the kidney. The mechanism appears to be one associated with secretion.

Horizontal sections of these ciliated borders show that the cilia are in many instances more of the nature of vertical plates than filaments. Moreover, the peripheral, more conspicuous cilia (plates) of each group are simply extensions of the cell membrane, rendered more robust by adherent remnants of inter-cellular cement. In some cases the only discernible 'cilia' are the opposite lateral parts of the cell membrane, brought to a point distally. Cross sections of such 'pairs of cilia' frequently show only an annular or elliptical structure, the thin wall of which represents a persistent cell membrane projecting beyond the level where the cytoplasm has disappeared in the process of secretion.

The basal granules likewise are not genuine basal corpuscles, comparable to those of the ciliated cell of the ductuli efferentes, but simply nodal thickenings in the spongioplasm at the level where these non-motile cilia spring from the distal border of the cells. These 'cilia' are in fact spongioplasmic extensions of the cytoplasm, forced into rectilinear vertical membranes by the cytodistal accumulation of escaping secretion. They are in fact, as Fuchs (1902) described them for the human epididymis, prolongations of the filar mass of the protoplasm.

DISCUSSION

There is a striking similarity between the cytology of the cells of the ductuli efferentes of the rat and those of the gills of *Cyclas*¹ as described by Helvestine. The mitochondria have a very

¹This material has since been identified for us by Dr. Paul Bartch of the Smithsonian Institution of Washington as *Pisidium abditum* Haldeman.

similar form and distribution. The younger cells have the longer and more distinct cilia, and larger more conspicuous basal corpuscles. In the older cells the basal bodies are minute granules and have lost their intense staining capacity. In the case of *Pisidium* ('*Cyclas*') the factor contributing, with age, to the decreased length of the cilia, was thought to be abrasion incident to the more exposed position of these older cells on the marginal surface of the gills. In the case of the cells of the ductuli efferentes of the rat the analogous contributing factor is probably initial secretory activity.

Two prominent countervailing data to the theory of Henneguy and von Lenhossek that the basal corpuscles are partition products of the centrosome, and to its corollary that such cells must therefore divide by amitosis, are the observation of Saguchi that the ciliated cells of invertebrates may divide by mitosis, and that by Jordan that in the epididymis of the stallion the ciliated cells (which were interpreted as possessing genuine basal corpuscles) occasionally divide by mitosis. Helvestine has shown, however, that in *Pisidium* the branchial cells only divide by mitosis before cilia have developed. Moreover, Saguchi himself states that before undergoing mitosis the ciliated cells of invertebrates lose their cilia. As to Jordan's observation it now appears, in the light of the data derived from the study of these non-motile cilia of the epididymis, that these cells do not possess either true cilia or genuine basal bodies. Adult material gives the seemingly definite appearance of distinct cilia and true basal corpuscles, but the study of the development of these structures forces the conclusion that the filaments are not analogous with true cilia and that the attached granules are not genuine basal corpuscles.

To demonstrate mitosis, therefore, in these cells in no way invalidates the Henneguy-Lenhossek theory. Mitoses are in fact quite frequent in the cells of the epididymis of young individuals. Since these cells have not contributed their centrosome to the formation of the basal bodies and the cilia, they retain their capacity for mitotic division until fully differentiated for secretory activity. The subsequent extensive amitosis must

then be here explained as the result of the high specialization of these cells along the path of secretory activity.

To determine whether these cilia are actually non-vibratile we dissociated cells of the epididymis of the rabbit by teasing tissue in normal salt solution to which potassium hydroxide was added in the proportion of 1 to 1000. No motility could be discerned under these conditions.

Saguchi claims to be able to demonstrate the origin of cilia from mitochondria. The present study seems to give conclusive evidence negating any such claim. In the one technic employed only basal bodies were preserved (figs. 1 to 4); in the other, only mitochondria (fig. 5). The technic which revealed the mitochondria showed a relatively wide distal area free from mitochondria (fig. 5). This is the area occupied by the very conspicuous bacillary basal corpuscles as revealed by the alternate technic (fig. 2). The facts 1, that the ciliated cells of the ductuli efferentes do not divide by mitosis; 2, that no centrosome can be positively demonstrated apart from the collection of basal corpuscles; and 3, the similarity of the group of basal bodies before alignment along the distal margin to the fragmented centrosome of certain cells, as, for example, the giant cells ('megakaryocytes') of red bone-marrow, support the doctrine of the origin of the basal corpuscles of ciliated cells from a centrosome.

SUMMARY

1. The cells lining the ductuli efferentes of the epididymis of the white rat possess vibratile cilia attached to conspicuous bacillary basal corpuscles. The latter are centrosomal derivatives. Originally all of the cells are ciliated. Coincident with the assumption of secretory activity, both cilia and basal bodies begin to disappear. During their ciliated phase the cells divide only amitotically. The mitochondria are not genetically related to either the cilia or the basal corpuscles.

2. The cells lining the duct of the epididymis also apparently possess cilia attached to basal granules. The cilia however only represent persistent distal portions of the cell membrane, and, in small part, spongioplasmic remnants of the distal cytoplasm of

the cell. The basal granules represent terminal bars and nodal thickenings at the levels where the spongioplasmic filaments pass into unaltered cytoplasm. The non-vibratile cilia of these cells are the result of the disappearance of the hyaloplasmic constituents along the distal border during secretory activity. During their younger stages the cells divide mitotically, subsequently amitotically.

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PLATE 1

EXPLANATION OF FIGURES

1 Non-ciliated portion of wall of a ductulus efferens of 41-day old white rat showing stages in the formation, dispersion and migration of the basal bodies. The epithelium is in general two-layered. The upper cell at the right shows four deep-staining granules, the next two show large numbers of similar bodies, the fourth cell shows these granules arranged in two tiers, the distal granules being now bacillary in shape. The terminal bars also are conspicuous throughout. Note that two of the basal corpuscles have sprouted cilia. Flemming fixation, iron-hematoxylin stain. Drawn with a Leitz $\frac{1}{6}$ oil immersion lens, no. 10 ocular (B & L) and an Abbe camera lucida. Mag. $\times 1500$.

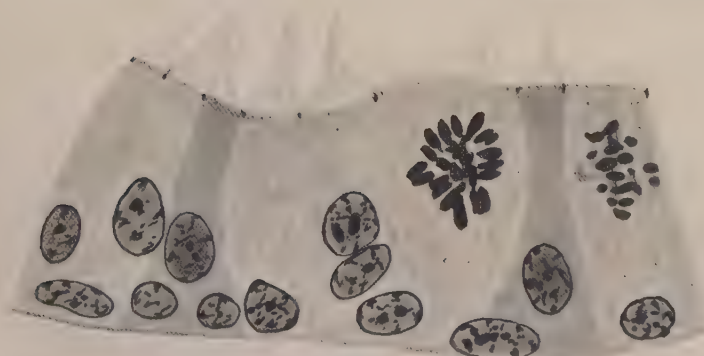
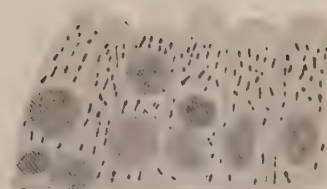
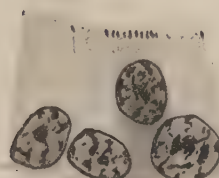
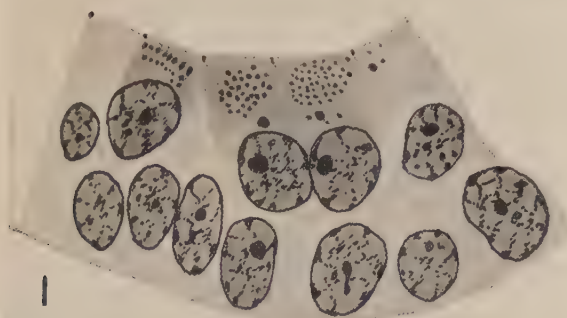
2 Later stage in ciliogenesis from same individual. Two of the cells have conspicuous bacillary, or dumb-bell-shaped, basal corpuscles, with long unmatted, presumably vibratile cilia. The cell at the left is at a stage preceding the presence of cilia. The cell at the right shows a stage in regression where the cilia are much shorter, somewhat clumped, and the basal corpuscles are no longer rod-shaped nor deeply staining. In the second cell from the right three granules have not become included among the basal bodies. $\times 1500$.

3 Portion of wall of ductulus efferens from same 41-day old rat. Six ciliated cells are shown, three with long, more distinct cilia attached to conspicuous rod-shaped basal corpuscles and three with much shorter, clumped cilia attached to inconspicuous lightly stained granular basal bodies. $\times 1500$.

4 Similar collection of four cells, the one with long cilia and deeply-stained bacillary basal corpuscles having a deeper-staining cytoplasm and nucleus. $\times 1500$.

5 Portion of wall of ductulus efferens from same 41-day individual, showing the character and distribution of the mitochondria. The two middle cells have long distinct cilia. The cell on the left has short, clumped, indistinct cilia. The distal border remains free of mitochondria. The technic does not preserve the basal corpuscles or terminal bars. Zenker-formol fixation, iron-hematoxylin stain. $\times 1500$.

6 Portion of wall of ductus epididymidis of same 41-day rat. The cells are all apparently ciliated. The 'cilia' are attached to small spherical, lightly staining basal granules. Two of the ciliated cells are in mitosis. Two other cells have a deeply-staining cytoplasm and nucleus. Note resemblance of ciliated border of cell at right to striated border on non-ciliated columnar intestinal cells. Note also that the lateral 'cilia' of the group of each cell are continuous with, actually extensions of, the terminal bars of intercellular cement. Transverse sections of these ciliated borders show that the cilia are actually vertical plates, and that the 'basal corpuscles' are nodal thickenings in the spongioplasmic network. Flemming fixation, iron-hematoxylin stain. $\times 1500$.



Resumen por H. J. Corper, por los autores O. S. Kretschmer y
A. C. Starry.

Venas pulmonares varicosas debidas a una
arteria anormal.

Los autores han hallado una arteria anormal en la porción inferior del lóbulo inferior del pulmón derecho de un perro, la cual era la causa de la presencia de numerosas venas distintamente dilatadas en la misma vecindad. Las venas irradiaban en forma de abanico hacia el hilus, aumentando de tamaño hacia arriba y terminando superficialmente en una línea de demarcación distinta, situada unos 2 cm. del ápice, alrededor del lóbulo inferior del pulmón. La arteria anormal era indudablemente de origen congénito. Las venas varicosas se debían aparentemente a la ausencia de capacidad de la corriente venosa ordinaria a través del hilus para transportar la cantidad excesiva de sangre aportada a esta porción del pulmón por la irrigación sanguínea doble, no existiendo corriente correspondiente para compensar la irrigación arterial anormal.

Translation by José F. Nonidez
Cornell Medical College, New York

PULMONARY VARICOSE VEINS DUE TO AN ANOMALOUS ARTERY

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ONE PLATE (TWO FIGURES)

Varicose veins have been described as being very common in certain localities of the body, especially the pendant portions, i.e., the lower limbs, but they can apparently exist in practically any portion of the body in which permanent venous stasis can occur as a result of anomalous or pathological conditions. In conjunction with pathological conditions associated with or in the lungs localized varicosities have been seen and described, and to them has been attributed the tendency to hemoptyses noted in certain diseases, most conspicuously pulmonary tuberculosis. Anomalous pulmonary varicose veins on the other hand are apparently extremely rare, since a survey of the literature available did not reveal a single case of this type described. During the postmortem examination of a dog not more than two years of age, which had been used in experimental tuberculosis studies, upon opening the chest there was noted in the lower portion of the lower lobe of the right lung quite a number of distinctly dilated veins. The veins radiated fan-like towards the hilus of the lung, the size increasing toward the hilus, and superficially terminated in a distinct line of demarcation about two centimeters from the tip around the lower lobe of the lung. Unfortunately the lung was removed and placed in formalin to be studied subsequently. Dissection of the lung revealed that there was an anomalous artery entering the lower right lobe at the tip where the varicose veins were situated. Since only the lungs remained for study it was impossible to trace the origin of the anomalous artery. The artery ran in the

lung substance from the tip of the lower lobe of the right lung decreasing in size upward for about 2 centimeters and supplying one of the main bronchi of this lobe. The artery was of considerable size (about 1 to 2 millimeters in diameter) as it entered the lung parenchyma and was about 2 centimeters long, terminating in the vicinity of the upper border of the venous varicosities.

The anomalous artery was undoubtedly congenital in origin because its size, the complete absence of all local inflammatory signs, and the distinct indentation or notching to the extent of about 5 millimeters at the point of entrance into the lung would hardly permit any other deductions. The varicose veins were apparently due to the inability of the ordinary venous outflow through the hilus to take care of the excessive blood supplied to this portion of the lung by the double blood supply, there being no corresponding outflow to compensate for the anomalous arterial supply.

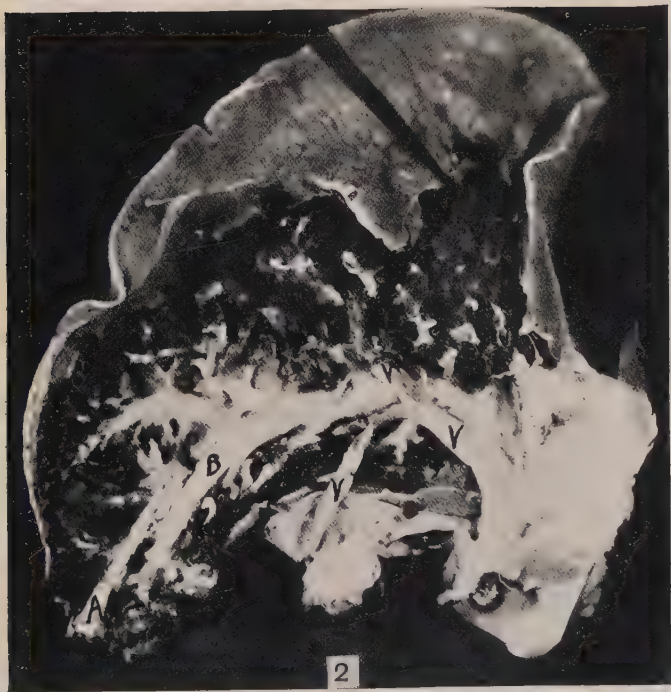
PLATE 1

EXPLANATION OF FIGURES

1 Posterior (dorsal) view of dog's lung showing varicose veins at posterior diaphragmatic aspect of the lower lobe of the right lung. The anomalous artery entered this lobe at the very tip where the veins converge and on the diaphragmatic side posteriorly.

2 Dissection of lung from mesial aspect in area of varicose veins showing the anomalous artery.

A = anomalous artery, B = bronchus, V = veins (dissected out, laid aside) coming from area supplied by the anomalous artery. There were two others of the same size coming from this area.



Resumen por el autor, C. C. Speidel.

Los troncos nerviosos colectores caudales longitudinales de los peces elasmobranquios.

En la cola de la raya existen cuatro troncos nerviosos colectores longitudinales, situados dos a cada lado de la columna vertebral. El colector dorsal de cada lado está situado en un punto opuesto a la unión del arco neural y el centro de cada vértebra. El colector ventral está colocado opuesto a la unión del arco hemal y el centro de cada vértebra. Estos colectores están alimentados regularmente por los nervios espinales de acuerdo con el siguiente sistema: La raíz ventral de la médula espinal se une con la raíz dorsal que emerge del orificio vertebral siguiente. De esta unión arrancan dos ramos principales, uno dorsal que se une con el colector dorsal y otro ventral que se une con el colector ventral. Estos ramos envían ramas al órgano eléctrico, que también las recibe de la raíz ventral. Los colectores mencionados envían ramas a los músculos y la piel, pero no al órgano eléctrico. La presencia de pequeñas ramas del colector ventral a los vasos sanguíneos del canal hemal y la presencia, a veces, de estructuras periféricas ganglionares indican la existencia de un aparato simpático primitivo. No existen ganglios a lo largo de los troncos colectores, ni tampoco existen fibras nerviosas ameduladas. Los colectores no son probablemente homólogos de los troncos del simpático de los vertebrados superiores, ni tampoco poseen una función especial en conexión con los órganos eléctricos. Suministran sin embargo, un mecanismo nervioso que hace más efectiva la coordinación de las variadas actividades de la cola. Nervios longitudinales caudales semejantes existen así mismo en el perro de mar y en el tiburón.

Translation by José F. Nonidez
Cornell Medical College, New York.

THE CAUDAL LONGITUDINAL COLLECTING NERVE- TRUNKS OF ELASMOBRANCH FISHES

CARL CASKEY SPEIDEL

Department of Anatomy, University of Virginia

ONE PLATE (TWO FIGURES)

The most recent description of the complete nervous apparatus of the electric organs in the tail of the skates, *Raiidae*, is the work of Ewart, ('92). The electric organs in the skate extend throughout the tail. They are long and spindle-shaped, lying on each side of the vertebral column, being homologous to and occupying the position of the middle (lateral) row of muscle cones that are present in other Elasmobranch fishes. In the diagram given by Ewart, the electric nerves are represented as a series of branches given off by each of the ventral roots of the spinal nerves. He also concludes that the electric motor nerve-cells in the spinal cord are certain large cells in the anterior horn resembling nerve cells except that they are much larger, ('93).

When Dahlgren first described the enormous irregularly-shaped cells located also in the anterior horn of the caudal portion of the spinal cord of the skate (3), it seemed uncertain whether or not Ewart had found the true electric motor nerve-cells. The writer, being engaged in determining whether or not these cells of Dahlgren had any nervous function in connection with the electric organs, undertook a thorough dissection of the skate tail to see exactly what the nerve relations were.

The larger barndoor skate, *Raia laevis*, which is plentiful during the summer along the coast of Maine, was selected as the most suitable species available for this work.¹ In a skate's tail which was first macerated for several days in 10 per cent nitric acid and then dissected, the nerves and their relations could be readily worked out.

¹ The material for this work was collected at the Harpswell Laboratory, Maine.

The result of the dissection together with other considerations led the writer to agree with Ewart that the large nerve-cells in the anterior horn throughout the tail resembling muscle motor nerve-cells except in size were the true electric motor nerve-cells ('19). Ewart's figure of the nerves and their relations, however, though correct as far as it goes, by no means tells the whole story. No mention is made of four prominent longitudinal collecting nerve-trunks close to the vertebral column which extend throughout the tail.² Nor in the literature dealing with the nervous system of Elasmobranch fishes has the writer been able to find any reference to these collecting nerves. This seems all the more surprising in view of the many studies that have been made upon the nervous system of these fishes, particularly upon the skate and dogfish.

The position of these longitudinal collecting nerve-trunks and their relation to the spinal nerves may be seen in the figure (fig. 1). The dorsal collecting nerve-trunks, or 'dorsal collectors,' are placed close to the vertebral column on each side near the junction of the neural arch and centrum. The ventral collecting nerve-trunks, or 'ventral collectors,' are also located close to the vertebral column on each side, near the junction of the haemal arch and centrum. Both dorsal and ventral collectors extend parallel to the vertebral column from the root of the tail to the tip of the tail.

There is one pair of spinal nerves for every two vertebrae. The nerve distribution is, therefore, bimeric in the tail, one nerve unit occurring every two segments.³ The junction of dorsal and ventral roots takes place well outside of the vertebral column. Each dorsal root unites with the ventral root which leaves the vertebral column one vertebra anterior to it. From this junction are given off two sub-branches which go to the electric organs and two main branches, a dorsal ramus and a ventral ramus. From the ventral ramus several additional

² The writer learned from Prof. U. Dahlgren that a longitudinal nerve in the skate tail had been seen by Dr. M. J. Greenman.

³ In the posterior portion of the tail the distribution becomes somewhat irregular, one nerve unit occurring every three or four or more vertebrae.

branches may be given off to the electric organ. This ramus then joins the ventral collector, at a distance of about three vertebrae caudad to the junction of the ventral and dorsal roots. The dorsal ramus also extends caudad for a distance of three vertebrae and joins the dorsal collector. In its course it usually gives off a single branch to the electric organ. From the ventral root just before this junction with the dorsal root, one branch (sometimes two branches) is also given off to the electric organ.

From the dorsal and ventral longitudinal collectors are given off branches to the muscles, skin, connective tissue, blood vessels and glands. In each nerve unit (i.e., every two vertebrae) two branches from the dorsal collector supply the dorsal row of muscle cones, and a third longer branch travels outward between the dorsal muscle cone and the electric organ, and ramifies in the connective tissue surrounding the electric organ. None of the sub-branches of this nerve actually supplies the electric organ itself, although at first sight this seemed to be the case. Likewise, in each nerve unit two branches from the ventral collector go to the ventral row of muscle cones. A third longer branch courses between the ventral muscle cone and the electric organ and ramifies in the skin and connective tissue laterad to the electric organ. In addition some smaller branches were found that appeared to penetrate the vertebral column to supply the blood vessels of the haemal canal. A few small peripheral ganglion-like structures were found in some places where two nerve branches joined or crossed. Not enough of these were found, however, to justify the conclusion that they occur regularly, and they are not shown in the diagram.

Since the longitudinal collectors coincide in antero-posterior extent with the electric organs, it was thought possible that they might be present in the skate only, as a parallel adaptation to the presence of electric organs. Upon examination of the tail of the spiny dogfish, *Squalus acanthias*, which has no electric organs, however, longitudinal collectors similarly placed were found. These extend from a point about two inches anterior to the second dorsal fin (i. e. the one nearer to the tip of the tail) to the caudal end of the vertebral column.

In the dogfish the nerve distribution is also bimeric as in the skate, each dorsal root uniting with the ventral root one vertebra anterior to it. The relation of the collectors to the spinal nerves, however, is not quite the same. From the junction of ventral root and dorsal root come two main rami as in the skate, but instead of one going to the ventral collector and one to the dorsal collector, both were found to go to the ventral collector, joining it two or three vertebrae caudad to the ventral root-dorsal root junction (fig. 2). A branch from the ventral root joins a similar branch from the dorsal root and the nerve thus formed extends caudally for a distance of about eleven vertebrae and then joins the dorsal collector. The collectors send branches to the muscles, skin and other structures. Further details were not worked out in the dogfish.

The question, of course, arises as to the exact function and significance of these collecting nerve trunks. Before the complete dissection was made it was thought possible that in the skate the collectors might be part of a mechanism for bringing about the discharge of all parts of the electric organ at one time. But the collectors were found to send no branches to the electric organ. The electric nerves, as has been pointed out, come from the ventral root and the ventral and dorsal rami. It is not probable, therefore, that the collectors are concerned at all with the electric organs.

Dr. J. S. Kingsley made the interesting suggestion to the writer that the arrangement of the nerves in the skate resembled somewhat the sympathetic apparatus in higher animals. The presence of longitudinal trunks parallel to the spinal cord with definite regular branches from each spinal nerve, the presence of small branches, from the ventral collector especially, that apparently go to the blood vessels, and finally, the occasional presence of peripheral ganglionic structures all invite comparison with the typical sympathetic system of higher vertebrates. Might not the Elasmobranchs have developed a separate sympathetic system for the tail?

Both in the skate and dogfish the longitudinal collectors are exclusively tail structures. It is known that in the trunk region

of Elasmobranch fishes a sympathetic system is present but it is rather poorly developed. In the head it is not represented except for the ciliary ganglion. The sympathetic ganglia first appear in development after the dorsal and ventral roots have united to form the spinal nerve-trunks just at their point of union, each ganglion containing from the first both afferent and efferent elements. A definite sympathetic cord connecting the separate ganglia in the trunk region is not developed in Elasmobranchs, although sometimes several ganglia may become joined together, while others may disappear at an early stage. Johnston states that these ganglia may extend for some distance into the tail.

It is known that some of the functions which in higher vertebrates are performed by the sympathetic system are performed in Elasmobranch fishes by the regular visceral efferent nerves. It is undoubtedly true that the longitudinal collectors are concerned in the performance of some sympathetic functions. The ventral collector in all probability contains fibers which supply the walls of the blood vessels of the haemal canal and which carry the impulses for the size regulation of these vessels. But in addition it also has its regular somatic motor and sensory elements. There are no ganglia occurring along these collecting trunks such as occur along the regular sympathetic trunks of higher vertebrates, as well as along the rudimentary sympathetic cord of the trunk region of the Elasmobranchs. Furthermore, the collectors contain only medullated nerve-fibers. Even the small branches of the ventral collector which pierce the vertebral column to go to the blood vessels of the haemal canal contain no non-medullated fibers. Sections of these branches after osmic acid fixation show that the fibers are without exception medullated. Any sympathetic functions, therefore, that are carried on by the collectors and their branches are performed by regular medullated nerve-fibers.

It seems to the writer that these caudal longitudinal collecting nerve-trunks are not in any way homologous to the sympathetic cords of the higher vertebrates. Their arrangement does, however, afford a nervous mechanism which probably makes for more efficient coördination of the various activities of the tail.

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PLATE 1

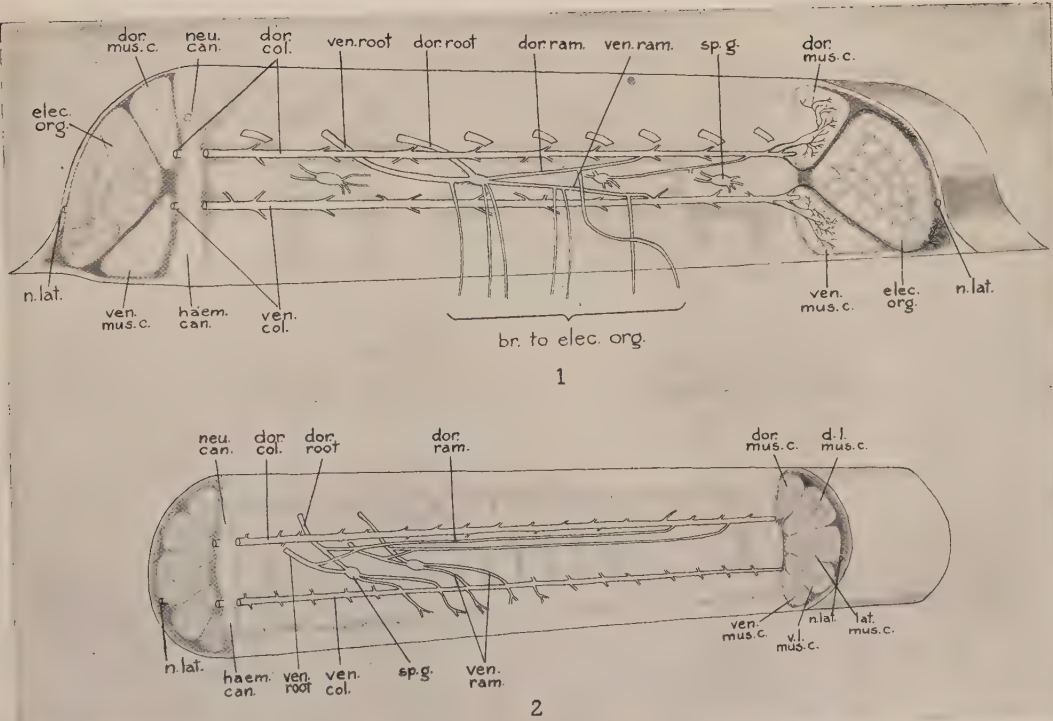
EXPLANATION OF FIGURES

1 Stereodiagram of a portion of the tail of the barndoor skate, *Raia laevis*. On one side the skin, muscles and electric organ have been dissected away to show the ventral and dorsal longitudinal collecting nerve-trunks and their relations to the spinal nerves and their branches. Four longitudinal collecting trunks may be seen, two on each side of the vertebral column. Only one nerve unit is shown in detail. One ventral and one dorsal root occur every two vertebrae. From their junction comes the dorsal ramus which goes to the dorsal collector, and the ventral ramus which joins the ventral collector. Note that the branches to the electric organ all come from the ventral root, dorsal and ventral rami, none from the dorsal and ventral collectors.

2 Stereodiagram of a portion of the tail of the spiny dogfish, *Squalus acanthias*. On one side the skin and muscle has been removed to show the longitudinal collecting nerve-trunks and their relations. As in the skate there are four longitudinal collecting trunks, two on each side of the vertebral column. Two nerve units are shown. Note that the nerve branches are somewhat different from those of the skate. From the junction of ventral and dorsal roots come two ventral rami which join the ventral collector. A branch from the ventral root joins a branch from the dorsal root forming a dorsal ramus which, after extending caudally for about ten vertebrae, joins the dorsal collector.

ABBREVIATIONS

- | | |
|--|--|
| <i>br. to elec. org.</i> , branches to the electric organ. | <i>lat. mus. c.</i> , lateral row of muscle cones. |
| <i>dor. col.</i> , dorsal collecting nerve-trunk (dorsal collector). | <i>neu. can.</i> , neural canal. |
| <i>dor. mus. c.</i> , dorsal row of muscle cones | <i>n. lat.</i> , nervus lateralis (lateralline nerve). |
| <i>d. l. mus. c.</i> , dorso-lateral row of muscle cones. | <i>sp. g.</i> , spinal ganglion. |
| <i>dor. ram.</i> , dorsal ramus. | <i>ven. col.</i> , ventral collecting nerve-trunk (ventral collector). |
| <i>dor. root</i> , dorsal root. | <i>ven. mus. c.</i> , ventral row of muscle cones. |
| <i>elec. org.</i> , electric organ. | <i>v. l. musc. c.</i> , ventral-lateral row of muscle cones. |
| <i>haem. can.</i> , haemal canal. | <i>ven. ram.</i> , ventral ramus. |
| | <i>ven. root</i> , ventral root. |



Resumen por el autor, W. C. George.

Nota sobre la circulación pulmonar de los
vertebrados.

El autor clasifica y discute los tipos de anomalías hallados en las arterias y venas pulmonares del hombre, bajo el punto de vista de su origen embriológico y de sus homologías probables con la circulación de la vejiga natatoria de los peces.

Translation by José F. Nonidez
Cornell Medical College, New York

A NOTE ON THE PULMONARY CIRCULATION IN VERTEBRATES

W. C. GEORGE

Department of Anatomy, University of North Carolina

A study of the anomalies that occur in the pulmonary circulation of man and the vertebrates is of value not only from the standpoint of their early embryonic origin, but also for the bearing that they have on the evolutionary origin of the respiratory apparatus of air-breathing vertebrates. It is now generally accepted that most vascular anomalies can be interpreted as being enlarged survivals of embryonic blood vessels that normally atrophy or remain inconspicuously small. Many are of only slight anatomical interest, but that some of them are not mere haphazard survivals seems clear when one notes their correspondence to typical conditions in lower forms. Many of the recorded anomalies of the pulmonary circulation fall into this significant group.

ANOMALIES IN VEINS

In a previous paper ('22) I reported an anomaly in the human pulmonary circulation in which the upper left lobe of the lung was drained by a vein opening into the left innominate vein and so into the systemic circulation. Similar anomalies have been reported by Blair ('02), Thane ('06), Patterson ('13), and Nützel ('14).

A number of cases have been reported in which one or more of the right pulmonary veins open into the normal superior vena cava. McCotter ('16) cites twelve such cases reported by various writers and a number of similar anomalies in which the right pulmonary veins terminate in the right atrium. He states also that "the somewhat rare condition of a communication between the left pulmonary veins and the persistent left superior vena

cava or with a persisting portion of this channel has been reported eleven times. These vary according to the portion of the left anterior cardinal vein that remains patent."¹ E. A. Park ('12) reports a case in which the right pulmonary vein enters the posterior vena cava.

Unusual drainage paths for the lungs occur among lower vertebrates also. Warren ('98, '00) describes three cases in the frog in which there was a communication between the rectal branch of the hepatic portal vein and the apex of the left lung, another similar case in which the communication was with the right lung, and another case in which an artery and a vein ran side by side. The artery was a branch of the posterior mesenteric artery. The vein forked, one branch being continuous with the renal portal vein and the other with the rectal branch of the hepatic portal.

ANOMALIES IN ARTERIES

Abnormal channels in the pulmonary circulation are not limited to the venous system. Atypical pulmonary arteries are known among the lower animals and a number have been reported from human anatomy. McCotter ('10) describes an anomalous pulmonary artery arising from the lower part of the thoracic aorta and passing to the right lung. He cites nine other recorded cases of anomalous pulmonary arteries. In seven of these the anomalous arteries arose from the thoracic aorta, one was given off from the abdominal aorta and one from an intercostal artery. Cases, cited by Huntington ('19), have been reported of the left pulmonary artery taking its origin from the left subclavian artery or having anastomoses with it; of the right pulmonary artery arising from the innominate artery, and cases in which the right subclavian arose from the bifurcation of the pulmonary trunk.

Anomalous pulmonary arteries in the frog have been described by Mudge ('98) and by Warren ('00). In Mudge's case the caudal tip of the right lung was supplied by an artery arising from the coeliac artery and the caudal tip of the left lung was supplied

¹ The literature list of the original reports may be had by referring to McCotter's paper.

by two arteries arising from the superior mesenteric artery. These arteries were accompanied by veins that terminated in the portal vein. In the case of Warren, the anomalous pulmonary artery was a branch of the posterior mesenteric artery. Williams ('09) describes cases in *Necturus* in which an artery arising from the seventh intercostal, posterior to the coeliac artery, passes to the left lung.

It is of some interest to observe here that accessory pulmonary arteries occur normally in some reptiles. In several of the *Lacertilia* they arise from the aorta or from the mesenteric arteries. In the *Ophidia* they arise from the aorta or from some of its splanchnic branches (oesophageal, gastric, hepatic).

EMBRYOLOGICAL BASIS

The important work of Evans and others showing the origin of the blood vessels of the vertebrate body from early formed capillary networks furnishes the basis for an understanding of the relations and mode of origin of these anomalies as well as for the typical conditions in the vertebrate vascular system.

We have in the developing embryo three anastomosing systems of capillary nets that particularly concern us in an analysis of these vascular anomalies. They are the median splanchnic plexus around the gut and the two lateral cardinal plexuses. Evans ('09) has shown that the cardinal veins are first represented by delicate capillary plexuses, the anterior cardinals forming first and the posterior cardinals forming later as chains of capillaries arising from the ducts of Cuvier and growing caudally into the splanchnopleure. It seems evident that the associated subcardinals and supracardinals arise from a further extension and development of these lateral chains of capillaries. Schulte and Tilney ('09), in speaking of the formation of the posterior vena cava, say: "A venous plexus surrounding the aorta is antecedent to the formation of the trunk vessels. The variously named cardinals are merely dilated portions of this reticulum along the major hydrodynamic lines, which, responding to the larger volume of blood they transmit, dominate the picture. The smaller transverse channels were long treated as of little morpho-

logical importance, except where from external factors in the course of development, the flow became retarded in one of the longitudinal vessels, when they entered the field of consciousness under the term *anastomoses* as a means of accounting for the emergence or enlargement of another longitudinal line."

The changes in the posterior cardinal plexus in later development are shown in the figures of plates I and II of Lewis ('02) and text figures 8 to 11 of McClure ('06). The connections of the splanchnic plexus in the region of the developing lung bud have been worked out by Brown ('13), Squier ('16), and Huntington ('19). Connecting vessels extend between the median splanchnic and the dorsolateral cardinal plexuses. By placing side by side and so combining Huntington's plate V and Brown's plate I, one can get a clear picture of the connections of the splanchnic plexus with the aortic arches and the aorta on the one hand, and with the cardinal veins and the venous portion of the heart on the other. The persistence of some of these embryonic connections accounts for the typical minor connections existing between the vessels in the lungs and the body vessels, and the enlargement of certain unusual ones and the suppression of the normal paths in various individuals accounts for the variations and anomalies that we find in the pulmonary connections and also in other trunk vessels of the thorax and abdomen, e.g., the duplication of the lower portion of the inferior vena cava or the emptying of the inferior vena cava into the azygos vein.

TYPES OF PULMONARY ANOMALIES

We find the following general types of anomalies in pulmonary vessels resulting from the abnormal enlargement of one or another part of the embryonic vascular plexuses.

Veins. 1. Left lung drained by a vein opening into the innominate vein. This represents enlarged portions of the splanchnic and cardinal plexuses that normally become the left bronchial and accessory hemiazygos veins.

2. Right lung drained into the superior vena cava by a vessel that represents enlarged portions of a plexus that normally become the right bronchial and azygos veins.

3. Anomalous right pulmonary vein drains into right auricle. Here we have persisting, instead of a connection with the superior vena cava, an anastomosis between the embryonic pulmonary plexus and the heart.

4. Anomalous left pulmonary vein communicating with the anomalously persisting left superior vena cava. In these cases we have a condition essentially similar to (2), due to the persistence of the left duct of Cuvier.

5. In Park's cases in which the pulmonary vein enters the posterior vena cava we have the pulmonary plexus retaining a connection with the heart through that portion of the splanchnic plexus that normally becomes the hepatic portion of the posterior vena cava.

6. In those cases reported for the frog in which there was a connection between the vessels of the lungs and a branch of the hepatic portal vein, we apparently have enlarged and persisting a channel in the splanchnic plexus between the points of origin of the lung bud and the hepatic diverticulum.

Arteries. 1. Anomalous pulmonary arteries arising from the coeliac and mesenteric arteries represent persisting and enlarged connections between the pulmonary plexus and those branches of the dorsal aorta to the splanchnic plexus that become the coeliac and mesenteric arteries.

2. Anomalous pulmonary arteries arising from the dorsal aorta or from the intercostal or subclavian arteries represent persisting and enlarged connections between the embryonic pulmonary plexus and the dorsal aorta or an intersegmental branch of the aorta. Some of these doubtless represent anomalously enlarged bronchial arteries, connections with the vascular nets in the walls of the air-chambers having been effected through the bronchopulmonary capillary connections.

Considerable additional interest attaches to these anomalies when we compare with them the course of the blood supply of the air-bladder in fishes. There is much variation in the circulatory system of fishes. The arteries of the air-bladder are derived sometimes directly from the aorta, sometimes from the coeliac artery, and sometimes from the last efferent branchial artery.

Barrell ('16) points out that in the different groups of fishes there is considerable variation in the place of origin of the pulmonary artery, and that it is in closest relation to the arterial system of the stomach and intestines in those fishes to whom in their evolutionary history air breathing by means of the air-bladder has never been more than a secondary mode of oxygenation. In those forms in which air breathing is an important mode of oxygenation, the pulmonary arteries arise from the branchial arteries. Only in those forms in which they do so arise and in which the veins from the air-bladder empty directly into the heart do we find the vascular basis from which it was possible to evolve a circulation with separated systemic and pulmonary systems.

In most of the higher fishes, Teleostomi, the air-bladder is supplied with blood by branches of the coeliac artery, with the addition of small branches arising directly from the dorsal aorta. In the dipnoan *Protopterus* the pulmonary arteries arise from the two dorsal aortae between the point of their union to form the single dorsal aorta and the origin of the fourth efferent arteries. In the ganoid *Polypterus* and in the dipnoan *Neoceratodus*, however, the arteries for the air-bladder are derived from a branch of the last or fourth pair of efferent branchial arteries.

From the network of minute vessels in the walls of the air-bladder the blood passes in some fishes to the portal vein, in some to the hepatic vein, and in some directly to the heart. Among the higher fishes most of the veins from the air-bladder join the hepatic portal vein, but some of them open into the posterior cardinals. In *Polypterus* they join the veins leaving the liver. In *Neoceratodus* the veins from the air-bladder unite to form a single vessel, which opens as a separate vein into the left auricle, and in *Lepidosiren* also a common trunk enters the heart. Thus in the forms making the largest use of the air-bladders in respiration, more of the blood is sent from the bladder directly to the heart and less to the liver.

We have seen how the pulmonary circulation varies in the different groups of fishes, and it seems probable that within a

single species there is much variation here, as there is in other vessels. It is not possible at present to say definitely what are the factors that determine the distribution of blood vessels. Danforth ('16) states that "The dissection of a few specimens of almost any fish will serve to show how greatly the vessels vary. . . . It seems probable to the writer that distribution of blood vessels in the fish is primarily a functional matter, dependent largely upon the interaction of blood pressure within the vessel and various forces that are brought to bear upon its outer surface. . . . That the same scheme tends to prevail in a given species or in related species is due, on this assumption, to the constant relation of other organs rather than to an inherent morphological individuality of any given vessel."

RESUMÉ AND DISCUSSION

We find among fishes that the air-bladder is supplied with blood:

1. By branches arising from the branchial arteries. The typical mode of origin of the pulmonary arteries in the higher air-breathing vertebrates corresponds to this condition.

2. By branches of the coeliac artery and in addition branches arising from the aorta. Corresponding to this condition, we find anomalies consisting in man of pulmonary arteries arising from the aorta, and in the frog of pulmonary arteries arising from the coeliac and mesenteric arteries.

3. By branches from the radices aortae between the last efferent branchial arteries and the union of the radices to form the single dorsal aorta. Those anomalies in which the pulmonary artery arises from the subclavian artery correspond to this condition.

The air-bladder is drained of blood: 1. By veins that empty directly into the heart. The typical condition of the pulmonary veins in man corresponds to this arrangement in the fishes.

2. By veins that empty into the hepatic portal vein and in addition by veins that empty into the posterior cardinal veins. Anomalies in the frog in which the pulmonary vein empties into the hepatic portal vein correspond to the first part of this condi-

tion, and anomalies in man in which the pulmonary vein empties into the innominate vein correspond to the second part.

3. By vessels that join the veins leaving the liver. Anomalies in man in which the pulmonary vein joins the hepatic portion of the posterior vena cava correspond to this condition.

We have in the vertebrate embryo anastomosing vascular plexuses from which may be derived the different circulatory schema that we find in the fishes and the other vertebrate groups. Due to the action of what we rather vaguely designate hereditary factors, or perhaps, as Danforth suggests, on account of "the constant relations of other organs," we find the same general scheme tending to prevail in a given species. In the ancestral fishes the pulmonary circulation seems to have been not definitely established but quite variable. In the higher vertebrate forms in which the lungs have entirely replaced gill respiration, the pulmonary vessels follow rather definite paths. Somewhere in the phylogenetic history of these forms, as a result of some unknown factors, the existing typical course of the pulmonary circulation was established, and only rarely do we find a large part of the blood to or from the lungs following channels similar to those that are typical in fishes, but anomalous in adults of the higher vertebrates. To what extent we are justified in assuming the intervention of an hereditary factor in the production of these anomalies is uncertain, but it is nevertheless significant to note in the embryo the vascular basis from which the anomalies are derived, and to observe that in them we have the blood following channels similar to the channels that are typical for ancestral relatives in which the course of the pulmonary circulation is less definitely established.

The adoption of the comparative point of view with regard to anomalies may enable us to look upon them not as mere haphazard freaks of nature, but as atypical structures that fit into the body of organized anatomical knowledge.

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Resumen por el autor D. M. Rioch.

La morfología y comportamiento de la células emigrantes en los cultivos de tejido del bazo del pollo.

Con el nuevo énfasis que se ha puesto en el estudio de la célula viva, el autor cree que el cultivo de tejido del bazo del pollo ofrece muchas ventajas para la observación de las células sanguíneas. El crecimiento de estos cultivos tiene lugar en las células mesoteliales, mesenquimatosas y emigrantes. El autor clasifica a las células emigrantes en las siguientes categorías: a) células sanguíneas granulares; b) células sanguíneas no granulares; y c) macrófagos de tejido. Existen dos variedades de células granulares, una con gránulos redondos muy refringentes y otra con gránulos también muy refringentes pero largos. Un cuatro por ciento de los gránulos redondos son basofílicos, todos los otros gránulos son eosinofílicos. Las células sanguíneas no granulares comprenden los linfocitos y monocitos, los cuales pueden distinguirse entre sí por sus reacciones en las soluciones diluídas de colorantes vitales; los linfocitos adquieren unas cuantas vacuolas después de veinte o veinticinco minutos, mientras que los monocitos adquieren muchas más vacuolas casi inmediatamente. Las vacuolas se desarrollan también en otras células, asemejándose los granulocitos a los linfocitos, y los macrófagos a los monocitos. Las diferentes clases de células presentan tipos característicos de actividad amiboidea. Algunas veces el autor ha visto células que envían procesos libres en la solución y que abandonan el cubre-objetos, aparentemente mediante sus propias energías. La capacidad para fagocitar los gránulos de melanina es también característica de cada tipo celular, aunque en ningún caso se observan movimientos en las células efectuados con este propósito. La entrada del gránulo en la célula depende aparentemente de la viscosidad del citoplasma y del área de contacto entre el gránulo y la célula.

THE MORPHOLOGY AND BEHAVIOR OF THE MIGRATORY CELLS IN TISSUE-CULTURES OF THE CHICK'S SPLEEN

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FOUR FIGURES

INTRODUCTION

With the renewed interest in the study of living blood-cells, stimulated by the work of Lambert ('15), Evans ('15), Tait ('18), Fenn ('21), Prigosen ('21), and Sabin ('21), it seemed that tissue-culture offered a new method of approach for observations on the blood-cells of the spleen. For this reason I undertook a study of the behavior of the cells arising in tissue-cultures of the spleen from the chick embryo. However, it became necessary first to classify the cells, as the behavior of each type was characteristic for that type, especially in regard to the phenomena of locomotion and phagocytosis. My observations were in large part directed towards the rôles, if any, played by sensibility and by chance in these phenomena.

I wish to express my thanks to Mrs. W. H. Lewis and to Dr. W. H. Lewis, in whose laboratory this work was done.

MATERIALS AND METHOD

Cultures of spleen from chick embryos (seven to twenty days' incubation) were made in Locke-Lewis solution.¹ These were turned upside down and incubated at 37° C. Smears were made from cells obtained in cultures from older embryos and stained

¹ Locke-Lewis solution consists of 85 cc. Locke solution (NaCl, 0.9 per cent; KCl, 0.042 per cent; CaCl₂, 0.025 per cent; NaHCO₃, 0.02 per cent), 15 cc. chicken bouillon, plus 0.5 grams dextrose per 100 cc. of solution.

in Wright's blood-stain. Spreads of the spleen in solutions of neutral red and brilliant cresyl blue were also prepared. For the study of phagocytosis, melanin granules, obtained from a teased piece of retina, were selected as the foreign body, because they are not toxic, can be readily recognized, and are easily obtained sterile. Threads of the *Sporotrichium beuermanni*² and spores of *Lycopodium* were also used as foreign bodies.

GENERAL CHARACTERISTICS OF THE SPLEEN

In chicks of seven days' incubation the spleen is only a fold of the mesenchyme of the body cavity; it differentiates rapidly, however, so that at nine days it forms a distinct, reddish, spherical organ. At twelve days the color has changed to brownish red, the only change thereafter up to twenty days being an increase in size from approximately 1.5 mm. to about 4 mm.

A spread made from the whole spleen of a seven-day chick embryo showed that it consisted of several lobes of undifferentiated mesenchyme, separated by narrow sinuses, and was covered by mesothelium. The sinuses were crossed by numerous reticular fibers and contained a few lymphocytes and erythrocytes. The mesenchymal cells bordering the sinuses were joined together by short, broad processes, some of those at the edge being almost free, the others forming a sponge-like tissue. The nuclei of these cells were large, resembling those of the blood-cells; the cytoplasm was clear, except for mitochondria and some granules of yolk. Further to the interior of the mesenchymal lobes the cells were packed together, their outlines indistinguishable. In spreads made from a piece of the spleen of an eight-day embryo the sinuses were broader than at seven days and contained more blood-cells. The sponge-like tissue was broader and within it were a few cells containing large refractive round granules in their cytoplasm, like the granules of blood-cells. These cells were surrounded by others with clear cytoplasm, to which they were joined by short processes. In spreads made from nine-day embryos the sinuses contained numerous blood-cells of all varieties, and in the sponge-

² The culture of *Sporotrichium beuermanni* was obtained through the kindness of Dr. C. G. Guthrie.

like tissue, besides a number of cells with round granules, there were a few with highly refractive long granules in the cytoplasm. The mesenchymal lobes appeared somewhat smaller and more numerous than in the seven-day embryo. In spreads from the older chicks the sinuses were much broader and, except for an occasional narrow central channel, were filled with blood-cells of all kinds, though mostly of the granular varieties. The sponge-like tissue was broader also and the majority of the cells composing it contained specific round and long granules in their cytoplasm. The undifferentiated mesenchyme was limited to small islands, as it were, surrounded by the sponge-like tissue. In some of these preparations the edges of the cover-glass were not sealed and capillary currents resulted from the evaporation of the drop, in which the cells of the sponge-like tissue were swayed back and forth so as to leave no doubt but that they were joined together. Erythrocytes were seen to flow through narrow spaces almost as though they were drops of fluid, but the leucocytes seemed much more viscous and often stuck.

CULTURE OF THE SPLEEN

Carrel and Burrows ('11) observed that growth of spleen tissue could be obtained in tissue cultures. Lambert ('15) made cultures of chick spleen in a plasma clot, and described a profuse outgrowth of mesenchymal cells and the production of great numbers of blood-cells. I have found that the growth from the spleen tissue may be divided into two distinct groups: 1) mesenchymal and mesothelial cells; 2) free migratory cells, that is blood-cells and tissue-macrophages.

In my cultures from embryos of seven to nine days' incubation there were profuse growth of mesenchymal cells, frequent broad sheets of mesothelium, and some blood-cells, mostly of the non-granular variety. In growths from older embryos mesenchymal cells were seldom seen and only occasional detached mesothelial cells. Blood-cells, on the other hand, were present in great numbers and in all varieties. There were a few tissue-macrophages in cultures from embryos of all ages.

The mesenchymal cells were large and irregular in shape, with a medium-sized, oval nucleus and rather granular cytoplasm, in which could be distinguished rod-shaped mitochondria (straight and curved), granules that stained with neutral red, and sometimes a foreign body. These cells grew away from the explant in long radiating strands, migrating slowly over the cover-glass, so that in some cultures, after the explant had degenerated (three to four days), there still remained around it a halo of young mesenchymal cells which continued to grow away from it. The mesothelium grew out usually as a membrane, composed of oblong cells with somewhat smaller oval nuclei and cytoplasm containing a few mitochondria and sometimes one or two granules of yolk. In many of the cultures there were also present scattered mesothelial cells with very long, finely branching processes. These detached cells grew in the same manner as the mesenchymal cells.

It is perhaps worthy of note that few endothelial cells were observed in the spreads, nor did any typical outgrowth of these cells occur in the cultures. This appears to indicate that endothelium is not abundant in the spleen, for these cells grow profusely in cultures from tissues containing it, as has been shown by Dr. W. H. Lewis ('21).

TYPES OF MIGRATORY CELLS AND THEIR BEHAVIOR

The leucocytes and macrophages in the splenic pulp migrated out freely over the cover-glass and could be readily studied, both under normal and abnormal conditions, during long periods. After three or four days there were many dead blood-cells at the bottom of the drop, while both the blood-cells and the mesenchymal cells on the cover-glass remained in good condition.

Cullen ('03), in a study of the leucocytes in twenty-nine varieties of birds, classified them as follows: 1) small mononuclear leucocytes; 2) large mononuclear leucocytes; 3) eosinophiles with *a*) round eosinophilic granules or, *b*) eosinophilic, spindle-shaped bodies; 4) mast cells, with round basophilic granules. Schmeisser ('15) gives the same classification for the chicken's blood. Burnett ('17) considers the cells with the spindle-shaped

granules as the polymorphonuclears or neutrophiles, as he claims these granules have a slightly more purplish tint than the round granules which he calls eosinophiles. Doctor Sabin ('21), on the basis of the relative numbers of the cells in the blood, considers the round-granule cells as equivalent to the neutrophiles and the long-granule cells as equivalent to the eosinophiles of the mammalian blood. She classifies the non-granular cells as lymphocytes and monocytes. Danchakoff ('16) does not distinguish between the round- and the long-granule cells, treating both as granulocytes, though she pictures them both in her plates. She also finds small lymphocytes, large lymphocytes, and macrophages, besides hemocytes, in the normal chick's spleen. As it was not possible in the living cells to distinguish the basophilic from the eosinophilic granules, I have used the term 'round-granule' cell to include mast cells and the eosinophiles with round granules. The descriptive term 'long-granule' cell seemed preferable for the cell with eosinophilic, spindle-shaped bodies. I have found it practicable to divide the non-granular cells, on the basis of their morphology and staining properties, into three groups: *a*) lymphocytes, *b*) monocytes, and *c*) tissue-macrophages.

The round-granule cells (fig. 1) varied in size from cells as large as the near-by erythrocytes to small round cells half that size. The smaller ones were more nearly round, with filamentous pseudopodia. The larger cells showed more amoeboid movement, frequently becoming finger or dumb-bell shaped and triangular. The pseudopodia were broad and thick, though in the resting state the cells were always round. Occasionally a cell sent out a single extraordinarily long process, six or seven times its own length. The nucleus was more opaque than the cytoplasm and was practically always eccentrically placed. In the small cells the nucleus was either oval or round, but in the larger cells it was round, horseshoe, or polymorphous. The granules were highly refractive and numerous, moving about freely as the cell changed shape, with no definite arrangement except when the cell was quiet, when they became arranged in the form of a crescent around the centrosphere, on one side of the nucleus.

In smears stained with Wright's blood-stain the nuclei became a deep, purplish blue and the cytoplasm a clear, pale blue. In about 4 per cent of the cells the granules stained dark blue (basophiles), while in the remainder the granules stained brilliant red (eosinophiles). With neutral red the granules stained bright orange-red, and with brilliant cresyl blue they stained a deep blue. In fifteen to twenty minutes after the supravitality stained spreads were prepared, these cells began to develop small vacuoles, staining pink with brilliant cresyl blue and pink or brick-red with neutral red. Within thirty minutes the majority of the cells showed from three to five vacuoles. The vacuoles developed *de novo* in any part of the cytoplasm.

The long-granule cells (fig. 2) resembled the round-granule cells in size, shape, and appearance of the nucleus. The granules, themselves, however, were mostly spindle-shaped, though some varied from short rods to long, slender curved bodies. They were arranged radially about the centrosphere, even when the cell was in amoeboid movement. With Wright's blood-stain these cells stained similarly to the round-granule cells. With neutral red the granules stained orange-red, and with brilliant cresyl blue they became clear sky-blue; in neither case, however, did they stain as readily as the round granules. These cells also developed cytoplasmic vacuoles which stained with neutral red and brilliant cresyl blue in a manner apparently exactly similar to those in the round-granule cells.

The lymphocytes (fig. 3) varied greatly in size, from cells somewhat larger than the near-by erythrocytes to others only half as large. Their shape was regular. The nucleus was occasionally kidney-shaped, though usually round or oval, and at one side of it the centrosphere, several mitochondria, and sometimes three or four small vacuoles could be seen. With Wright's blood-stain the nucleus became deep blue and well defined, and the cytoplasm a clear, pale blue. In supravitality stained spreads these cells did not develop any vacuoles for the first fifteen minutes, but by thirty minutes the majority of them contained from five to eight vacuoles, whose staining reaction was similar to that of the vacuoles in the granular cells. They appeared first

as very small, pink-staining bodies, which usually increased in size and developed into vacuoles. For the most part the vacuoles were arranged directly around the nucleus, sometimes outlining it.

The monocytes were about the size of the larger lymphocytes. Their outline was regular, the nucleus was round, oval, kidney, or horseshoe in shape. Besides the mitochondria, the cytoplasm sometimes contained a few fine granules, especially opposite the

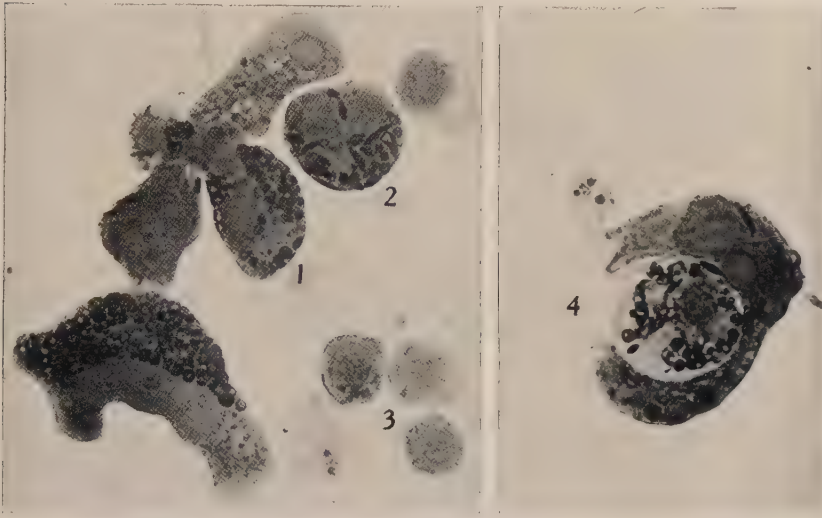


Fig. 1 Round-granule cell.

Fig. 2 Long-granule cell, in which most of the granules are spindle-shaped.

Fig. 3 Three lymphocytes.

Fig. 4 A macrophage spreading over a granulocyte.

concave side of the nucleus. In smears stained with Wright's blood-stain the nucleus became blue, but the color was not as deep or distinct as that of the lymphocytes. The cytoplasm stained a clear, pale blue. In supravitaly stained spreads, examined within ten minutes after the egg was opened, the monocytes were seen to contain from fifteen to twenty-five vacuoles and granules staining similarly to those in the lymphocytes.

The macrophages (fig. 4) were somewhat smaller than the average erythrocytes, irregular in shape, more or less oblong

with short sheet-like pseudopodia. The nucleus was oval and regular in outline, with from one to three distinct nucleoli. The cells usually contained two or three large fat-globules, one or more vacuoles, and sometimes debris from disintegrated cells. These cells were not distinguishable in smears stained with Wright's blood-stain. In supravitaly stained spreads they frequently contained large vacuoles, staining similarly to those in the other cells.

The thrombocytes, while not migratory cells, resembled lymphocytes so closely as to deserve mention here. They were about the same shape as erythrocytes, the cytoplasm was clear and contained a few granules at one end of the nucleus. No pseudopodia were sent out, but occasionally the cytoplasm was seen to flow sluggishly out on one side of the nucleus and then to return.

Vital dyes. In addition to the migratory blood-cells, the mesenchyme, mesothelial cells, and the erythrocytes developed granules and vacuoles which stained with the vital dyes. Prigosen ('21) described these vacuoles as accumulating in connective-tissue cells, as well as in chick and human blood-cells. She concluded that they are due to abnormal conditions of the cells and not to any specific action of the dye. Doctor Sabin ('21), working with the blood of the chick embryo, states that it takes about ten minutes for the granules to stain to their maximum capacity. She finds that the monocytes contain many more pink-staining bodies than do the lymphocytes, and that this constitutes an excellent criterion for differentiating these two types. Simpson ('21), working with rabbits, has also described the use of vital dyes in differentiating monocytes from lymphocytes. Evans and Scott ('21) have shown that the fibroblasts and macrophages of rats "exhibit a characteristic difference in their reaction to intra-vitam acid dyes," the former developing a few long vacuoles and the latter many round ones. All of these authors are agreed that it takes some time for the vacuoles and granules to appear. However, from my observations and also from those of Prigosen, it is evident that these bodies are not the result of a specific interaction of the dye and the cell, because

similar bodies developed in unstained preparations, while in certain of the stained preparations no vacuoles or granules were observed in the lymphocytes or granulocytes. Though brilliant cresyl blue and neutral red are called vital dyes, this is only a relative term, for when strong concentrations of these dyes are used for staining purposes the cells die immediately. It was also found that in all the cells, except the monocytes and macrophages, the number of granules produced was increased in more concentrated (more toxic) solutions of the dye. However, in no case, regardless of the concentrations of the dye used, was a lymphocyte seen to contain as many vacuoles as a monocyte.

Movement. The long-granule and the round-granule cells displayed striking amoeboid activity and rapid migration, but the non-granular cells, the lymphocytes, and the monocytes moved slowly. The tissue-macrophages were seldom seen far out on the cover-glass, but were usually around the edges of the explant and within the growth. They were not observed in active amoeboid movement, but their positions, relative to the explant, were such as to warrant the conclusion that they migrated out by their own energies. Maximow ('01) described them as the second type of cell to migrate between two cover-glasses which he buried in the tissues of rabbits.

The amoeboid movement of the granulocytes was a flowing of the entire cell. Usually the preceding edge was of clear cytoplasm, with several pseudopodia, sometimes fine and filamentous, sometimes broad and blunt, projected in front. Occasionally one or two granules were carried out in a pseudopodium. The pseudopodia were sometimes obliterated by the advance of the cell, at other times retracted, or, with a change in the direction of motion, they remained projecting from the side of the cell. At times the granules preceded, with no edge of clear cytoplasm in front of them; at other times, though more rarely, the nucleus was seen to go first. One part of the cell became fixed or moved very slowly, while the rest flowed out in front or came up from behind; e.g., the sides would become fixed and the central part of the cell flow out in front and then become fixed itself, or the nucleus would become fixed and the cytoplasm flow around it in

a new direction. However, the currents in the cytoplasm were very variable and followed no fixed patterns. These cells did not spread out and become flattened over the cover-glass, but projected and withdrew pseudopodia freely, so that at times there was considerable activity with little or no motion. Melanin granules adhering to the cell membrane were carried about on the ectoplasm and their position indicated that there was no rolling movement of the ectoplasm, as has been described by Jennings ('04) for the membrane of *Amoeba verrucosa*. Leo Loeb ('20) has described the amoeboid movements of the blood cells of *Limulus*. These cells send out a pseudopodium which is rarely retracted and which becomes rather stiff. The cytoplasm then flows into the pseudopodium and another is sent out in front. This is quite different from the movements of the granulocytes of the chick, in which the pseudopodia are freely retractable and the cytoplasm flows as a whole. In the blood-cells of *Limulus*, Loeb also describes the endoplasm, containing the granules, as being of a more viscous consistency than the clear exoplasm. No such differentiation could be made in the granulocytes of the chick.

The movement of the non-granular cells was similar to that of human lymphocytes, as described by Lewis and Webster ('21). The cells during amoeboid movement became elongated, the nucleus occupying approximately the middle portion of the cell. The preceding cytoplasm was spread out into two or three blunt pseudopodia, while the trailing edge of the cell was more spherical. As the nucleus and cytoplasm flowed into the advancing film of the cell, new pseudopodia were sent out in front. Sometimes pseudopodia were sent out and retracted and others sent out, without much movement of the cell as a whole.

Several times monocytes and macrophages were seen to remain stationary on the outgrowth from the explant or out on the cover-glass, projecting and withdrawing broad, blunt pseudopodia for long periods of time without these processes necessarily becoming attached to other cells or to the cover-glass.

Cells migrating through the outgrowth from the explant were seen to stretch themselves out along a fixed cell and then to

draw themselves forward; sometimes, in a group of several cells, one or two of them stretched themselves around the stationary cells as though trying to engulf them (fig. 4). In no case, however, was a living cell seen to be engulfed. Frequently a cell adherent to the cover-glass projected a pseudopodium down into the drop, following it with other pseudopodia until the area of cytoplasm remaining attached to the cover-glass was no longer enough to support the cell, which then left the glass and glided down to the bottom of the drop. In the process of leaving the glass the pseudopodia were distinctly seen to stretch out free into the solution, and the movement of the cell, rather than the force of gravity, caused it to leave the glass. In some cases the cell continued to thrust out and withdraw pseudopodia, even when the connection between it and the cover-glass was practically invisible, which is contrary to the statement of Ullman ('11). However, locomotion was only seen to occur when the cell was in contact with some surface between the solution and another medium, such as air, glass, foreign body, or another cell.

The presence of neutral-red vacuoles or of phagocytized foreign particles did not appear to affect the ability of the cells to migrate over the glass. In a hanging-drop of Locke-Lewis solution it was found that few of the blood-cells adhered firmly to the cover-glass, the majority of them either gliding or dropping off and sinking to the bottom of the drop, and in this way they differed markedly from mesenchymal outgrowth in the same culture, which was firmly adherent to the cover-glass and appeared spread out upon it. The contrast in the adhesiveness of the blood-cells and that of the mesenchymal cells, even when in the same environment, seems to indicate that there is an inherent difference in the stickiness of these cells as compared with those described by Lewis ('22). In this respect the blood-cells of *Limulus*, as described by Leo Loeb, and those of *Ligia*, described by Tait, resemble more closely the mesenchymal cells than the blood-cells of chick spleen, as they, also, were adherent and spread out on the cover-glass. When the slides had been placed upside down in the incubator for two or three hours, a large number of cells adhered to the glass and remained there for periods varying

from a few minutes to several hours after the slides had been righted for study under the microscope. It was noticeable that the older cells and those that had suffered traumata did not adhere to the cover-glass.

Phagocytosis. Observations upon the phagocytic power of the blood-cells were carried out for the greater part with melanin granules. In cultures in which a teased piece of the retina was grown with the spleen, some of the larger granular cells contained as many as five melanin granules, the smaller ones rarely any. The majority of the larger non-granular cells contained from five to ten pigment granules and some of the smaller ones as many as five. All of the macrophages contained granules, some of them so many as to appear almost black.

The most careful scrutiny failed to reveal any effort on the part of the blood-cells towards securing the granules. Pseudopodia were sent out in any direction, regardless of the proximity of the foreign bodies. Even when a granule bounced against the side of a cell, pseudopodia were projected as frequently from the opposite side as from that side. Feeding reactions, such as have been described for *Amoeba proteus* by Schaeffer ('16) and Kepner and Whitlock ('21), were not seen.

Granules in brownian movement were seen to strike against the side of the cells many times and to bounce off without adhering. At other times they became attached. Frequently a granule adhered to a cell by only one end, in which case it was carried about on the surface of the cell. Such granules have in several instances been watched for more than half an hour, during which time they were not taken into the cell, even though in one instance a pseudopodium was projected sheath-like on each side of the granule as if to engulf it.

No melanin granule was seen to be taken in unless it adhered to the cell throughout its entire length. When this occurred, however, or when a cell in amoeboid movement passed over a granule, the granule entered the cell. It seemed that a certain extent of surface contact was necessary before the granule was taken in; that is, when it had been wet by the cytoplasm it passed into the interior of the cell and the cytoplasm closed around it.

The only way of telling, in most cases, whether the granule was inside the cell or was still attached to the outside, was to observe whether or not it moved with the other cell granules, for, after a granule had entered a cell, it moved about with the cytoplasm and often remained unchanged for long periods. In other instances, within a few hours a small vacuole formed around it, as has been described by Smith ('21) for melanin granules and by Cross ('21) and Smith, Willis and Lewis ('22) for bacteria. In tissue-macrophages several granules were seen in brownian movement within a single vacuole, but in leucocytes the vacuoles were only slightly larger than the granules and there was usually only one granule in a vacuole. Within the vacuoles the granules are almost always in brownian movement. When the living culture was stained with neutral red the vacuoles stained pink. Digestion of the melanin granules was not observed.

Ledingham ('12), working with human leucocytes and bacteria in vitro, concluded that the establishment of contact between the cells and the bacteria was purely a matter of chance; that the entrance of the bacteria into the cells was not due to any amoeboid action of the cells, and that the phenomenon of phagocytosis followed the laws of adsorption. Sawtchenko ('15, '16), working in vivo and in vitro with the leucocytes of rabbits and guinea-pigs, states that there are two separate and essential conditions in phagocytosis: 1) the particle must become attached (coagglutinated) to the surface of the cell; 2) the cell must then wet the particle. The first phenomenon may take place without being followed by the second under certain conditions, e.g., at low temperature, but the second cannot take place without the first. He regards phagocytosis as a phenomenon of surface tension. Tait ('18), working with the blood of *Ligia* in vitro, postulated the relation between amoeboid movement and surface tension as follows: If a cell spreads out very thin over a surface with which it is in contact, it will phagocytize a particle of the same substance; but if it remains spherical when in contact with the surface of the substance, then it will not phagocytize the particle. He expresses the requirements in physical terms as the relation of the surface tensions between the cell, particle,

and solution. Fenn ('21), using the leucocytes from a peritoneal exudate from the rat in a suspension with carbon and quartz particles, showed that the rate of ingestion of the particles could be predicted by calculating the chances of collision between them and the cells. He also showed that the carbon was ingested more easily than the quartz and suggested that the cause of this might be identical with the cause of the greater tendency of the carbon particles to form clumps in solution. Smith ('21) has described the phagocytosis of melanin granules by cells in tissue-cultures of the embryo chick. My observations agree with his in regard to the ingestion of melanin granules.

When *Lycopodium* spores were introduced into the cultures, no giant cells such as those described by Lambert ('15) were formed, nor did the leucocytes congregate around the spores as he described, although cells were seen to migrate over the spores. The explanation of this difference in results may possibly lie in the culture medium, Lambert using plasma, whereas all of the above work was carried out in Locke-Lewis solution. On the other hand, giant cells were observed in two cultures, into neither of which had any foreign bodies been introduced. In one the giant cell seemed to be a mesothelial cell, with five nuclei; in the other there were six cells which seemed to be similar to the usual giant cells.

When a *Sporotrichium beuermanni* mycelium was added to the cultures, many of the threads were seen to be surrounded by five to ten leucocytes, and often it was difficult to decide from the appearance of the mass whether the leucocytes were fused together or not. However, occasionally a cell left the group and wandered away, indicating that there was no real fusion. Several times a cell was seen to stretch out along the full length of a thread, two or three times the ordinary length of the cell. Sometimes the cell left the thread and passed on, indicating that the thread had not been ingested; in other cases, however, a detached portion of a thread was seen in a vacuole within a cell.

Some cultures were made in a neutralized solution containing from 2 to 5 per cent of gelatin. The cells did not form vacuoles or granules, and so probably did not phagocytize any gelatin, as

has been described by Metchnikoff ('07, p. 108) when the gelatin is injected intraperitoneally into guinea-pigs.

SUMMARY

1. The migratory cells found in cultures of the spleen of chick embryos were of three types: *a*) granular blood-cells, *b*) non-granular blood-cells, and *c*) macrophages. The granular cells were of two varieties, one containing round granules, the other long granules. About 4 per cent of those with round granules took the basophilic stain; the remainder, as well as the cells containing long granules, were eosinophilic. Of the non-granular blood-cells the majority were lymphocytes, the others monocytes. The macrophages were distinguishable from the other types of cells by their large fat-globules, vacuoles, and debris.

2. With vital dyes the round granules stained intensely, while the long granules stained faintly. In dilute solutions of vital dye the monocytes usually showed numerous small vacuoles and granules, the macrophages numerous larger ones. The other cells did not show any vacuoles for fifteen minutes, but after that time a few appeared. When stronger solutions of the dye were used the number of vacuoles was greater. Vacuoles developed, however, when no dye was used.

3. The amoeboid movement exhibited by the granular and non-granular blood-cells and by the macrophages was, on the whole, characteristic for each type. Blood-cells were seen to send out processes into the solution and to leave the cover-glass, apparently by their own energies. Possibly, in a similar manner, these cells can cross fluid spaces in the body by leaving one point and dropping to another. Injured or old cells did not adhere to the cover-glass, but floated in the bottom of the drop.

4. Phagocytosis of melanin granules was not the result of any purposeful movements on the part of the cell; contact between the granule and the cell was a matter of chance. Once this had been established, two factors seemed to govern the entrance of the granule into the cell: 1) the entire length of the granule had to be in contact with the surface of the cell and, 2) the cytoplasm of the cell had to wet the granule.

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Resumen por el autor, G. W. Tannreuther.

Sistema urogenital anormal en un gato doméstico.

El autor describe un caso en el gato doméstico, en el cual el riñón derecho, ureter y vaso deferente faltaban. Los testículos eran del mismo tamaño, pero un estudio citológico ha demostrado que existían muy pocas células germinales en un estado ulterior al del crecimiento, en el testículo derecho. En algunos casos una célula ha pasado por la primera división del espermatocito sufriendo pronto una desintegración. El globus minor (epidídimo) del testículo derecho faltaba. El testículo izquierdo contenía células en todos los estados del desarrollo, desde la espermatogonia hasta el espermatozoide maduro.

Translation by José F. Nonidez
Cornell Medical College, New York

ABNORMAL URINO-GENITAL SYSTEM IN THE DOMESTIC CAT

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ONE FIGURE

The following anomaly as found in the domestic cat differs from any of those previously reported. The urino-genital system on the left side is complete and does not show any variation from the normal condition, except that the kidney is one-third larger than is usually the case in an animal of the same size. With the exception of the testis, all structures (kidney, ureter, and vas deferens) are absent on the right side.

In order to determine the exact condition of the genital structures, the inguinal canal was removed on both sides and the spermatic cord dissected apart. The left spermatic vein, artery, and vas deferens are present and normal as indicated in figure 1. Likewise the right spermatic vein and artery; but there is no trace of the right vas deferens. The testes as far as could be determined externally were similar in size. The testes were fixed in Bouin's fluid, imbedded in paraffin, and sectioned 12 μ in thickness.

The fibro-elastic capsule of the right testis was twice the thickness of that of the left. The histological structure of the seminiferous tubules and their supporting tissue was much the same in both. The greatest difference was found in the developing germ cells. The left testis contained sex cells in all stages of development from the spermatogonia to the mature sperm. The right testis, however, showed few cells that had progressed beyond the growth period. An occasional cell reached the first spermatocyte division, but appeared rather abnormal. The chromatin material was collected in a compact mass, irregular, and many of the cells gave evidence of disintegration. The spermatogonia, however, appeared normal.

The epididymis (globus major) showed a distinct continuity with the efferent ducts and testis proper. The histology of the epididymis did not show any departure from that of the normal. The globus minor portion of the epididymis from which the vas deferens takes its origin was absent.

Taking into consideration the sequence of development of the urino-genital system in the embryo, it is evident, since the epididymis (globus major) of the right testis is present, that the mesonephros (embryonic kidney) had reached a considerable degree of development before the anomaly started. The mesonephric duct (embryonic vas deferens), however, failed to develop, at least to establish connection with the cloaca, since there is no vestige of the vas deferens or ureter.

In studying the longitudinal sections of a 10-mm. cat embryo, it was found that the mesonephric duct was connected with the cloaca, but was solid at its posterior end and non-functional as an excretory tube. The mesonephric tubules, however, were well developed and showed completely formed glomeruli. The ureter had not begun its development.

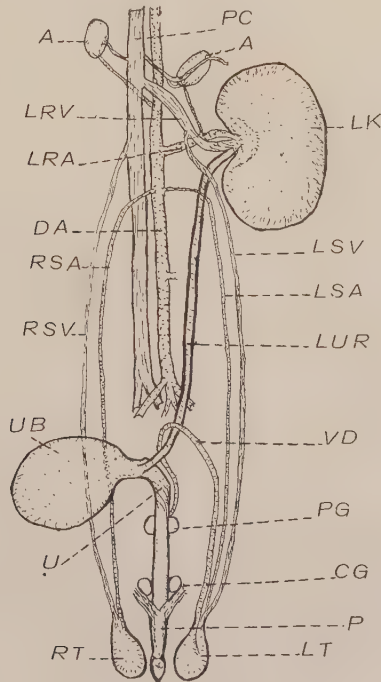
Judging from the conditions found in a 10-mm. cat embryo, the anomaly described above began at an early stage, at least at the time before the mesonephric duct established its connection with the cloaca.

The adrenal bodies and their blood supply were of the normal type. There was no trace of the right renal vein and artery.

Broek ('07) reported a human male subject, in which the whole right half of the urino-genital system was absent. Lyon ('17) described a similar condition in a female human individual which, however, possessed a ureter on the side of the body lacking the other urino-genital organs. Hunt ('18) reported the condition in a male cat in which there was no vestige of a right kidney, but in which the testis, vas deferens, and posterior end of the right ureter were present. Baillie ('22) described a case as found in the female rabbit, in which the right side of the urino-genital system was normal, except that the kidney was larger than usual. The left kidney, ureter, and uterus were absent, while the left ovary and the anterior end of the left oviduct were present.

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Figure 1. $\times \frac{1}{2}$ natural size.

ABBREVIATIONS

- | | |
|---------------------------------|----------------------------------|
| a., adrenal body | p., penis |
| c. g., Cowper's gland | p. g., prostate gland |
| d. a., dorsal aorta | p. c., postcava |
| l. k., left kidney | r. s. a., right spermatic artery |
| l. r. a., left renal artery | r. s. v., right spermatic vein |
| l. r. v., left renal vein | r. t., right testis |
| l. s. a., left spermatic artery | u., urethra |
| l. s. v., left spermatic vein | u. b., urinary bladder |
| l. t., left testis | v. d., vas deferens |
| l. ur., left ureter | |

REFERENCE MODELS OF THE PERITONEUM, FEMALE AND MALE

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TEN FIGURES

The models belonging to this series consist of two sets of casts. One set was made some years ago from the abdominal viscera of the female subject of which parts of the thorax were cast as Bellevue Model no. II, A.¹ It is entitled "Bellevue Model no. II, B, C, and D." The other set consists of casts of the anterior and posterior halves, respectively, of the interior of a frontally bisected abdomen from which most of the viscera were removed before the moulds were made. It is entitled "Bellevue Model no. IV" and was prepared from a colored subject that had succumbed to oedema of the larynx at the age of thirty-nine.

BELLEVUE MODEL NO. II, B, C, AND D

Cast II, B, of which the anterior and posterior aspects are illustrated in figures 1 and 5, was made as part of the routine preparatory to the casting of the pelvic diaphragm and viscera. The original objective was not attained on that occasion, however, owing to the fact that the subject's uterus was retroverted.

The cast of the abdominal viscera having been found to be very instructive, a further set of castings was planned, by which a three-dimensional view of all of the reflections of the peritoneum might be obtained. The viscera were accordingly returned to the posterior part of the mould and the larger part of the stomach and part of the liver and of the omentum majus were removed in order to display the interior of the omental bursa.

¹ Morrill, C. V., 1917. Reference model of the thoracic viscera, *Anat. Rec.*, vol. 12, p. 397.

The anterior aspect of the preparation was then remoulded and, the kidneys and suprarenals having been removed, a second mould was prepared from the posterior aspect also. The cast (II, C) made from the second mould is illustrated by figures 2 and 6.

In order to gain a three-dimensional view of the detailed relations of the mesentery and mesocolon, the viscera were later returned to the second mould, redissected and remoulded in such a way as to produce cast II, D, which is illustrated in figures 3 and 7. The half impressions, in the original mould, of the stomach, kidneys, and suprarenals were then completed. The separate casts of the kidneys obtained from the last two are illustrated in figure 3 and that of the stomach in figures 4 and 8.

The arteries of the subject had been injected several months before casting with equal parts of formalin and water, by which the viscera were sufficiently hardened to preserve their form throughout the duration of the entire operation.

The model as a whole has proved to be most instructive. Cast-B furnishes a posterior view of the viscera that is very rarely to be seen in a student's laboratory and reveals the wedge-like shape of the abdominal viscera as a whole, and the depth of the sagittal impression that accommodates the bodies of the lumbar vertebrae, the aorta, and the vena cava inferior. It also indicates in detail the manner in which the peritoneum is reflected from the viscera to the abdominal wall. Cast-C illustrates the relations of the omental bursa and of the foramen epiploicum and the peritoneal reflections about the hilus of the spleen. Cast-D affords a good illustration of the disposition of the mesentery and of the distribution of the jejunal and ileal branches of the superior mesenteric artery. The transverse colon and mesocolon are detachable in one piece and the route that should be followed by the surgeon in the operation of gastro-enterostomy is indicated with great clearness. The separate casts of the stomach (figs. 4 and 8), kidneys and suprarenals (fig. 3) are so arranged as to be attachable in situ.

The unusual features of the subject from which these casts were made include a low transverse colon (reaching to a point



Figs. 1 to 4 Bellevue Model no. II, B, C, and D, and the separately cast stomach, kidneys and transverse colon and mesocolon of a female subject, anterior view.

2 $\frac{1}{2}$ inches above the symphysis pubis); a right kidney that has dropped, as shown by the lateral inclination downward of the right renal vein, half an inch or more below the suprarenal; a retroverted uterus that has left an impression upon the part of the ileum contained in the pelvis, and an accessory hepatic artery that arises from the superior mesenteric.

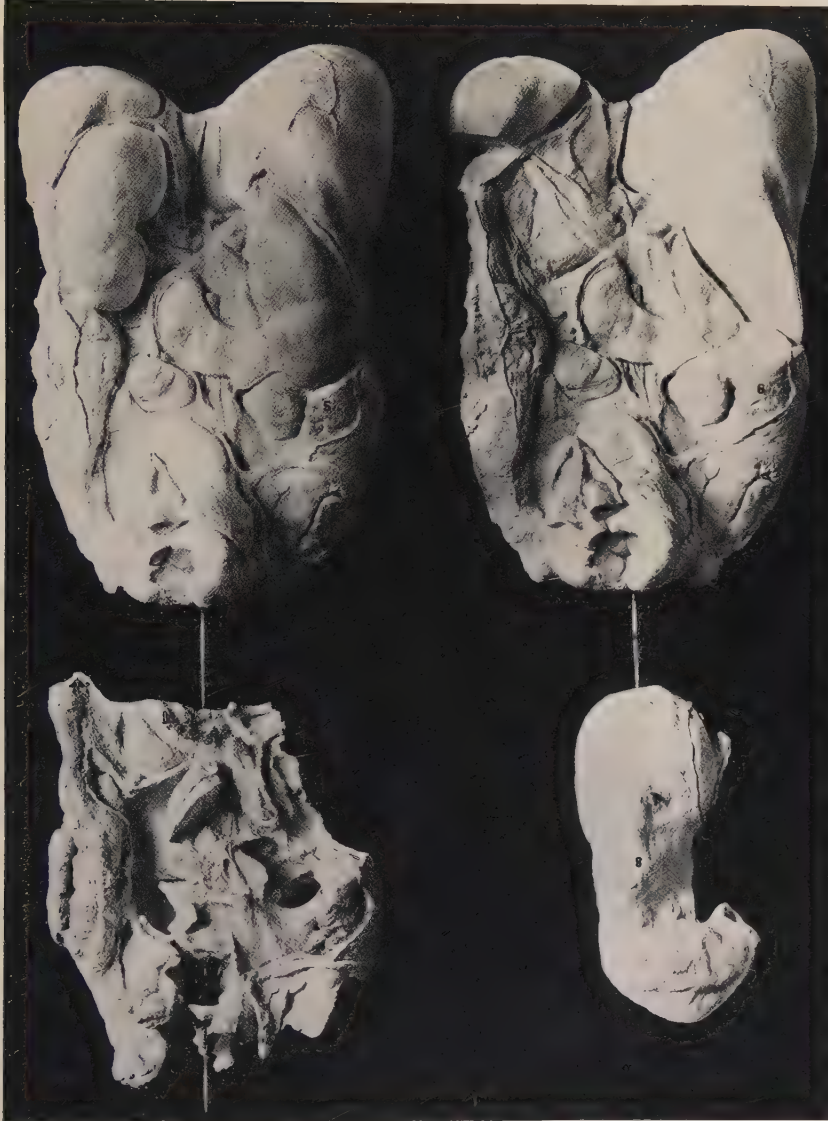
The accessory hepatic artery was removed from the proofcast as undesirable in a model of this kind, the size of the cast of the unusually small hepatic branch of the coeliac artery having been increased proportionately. The other unusual visceral features of this subject are reproduced without modification.

BELLEVUE MODEL NO. IV

This model consists of two plaques designed for hanging on the wall. The anterior half, illustrated in figure 9, shows, in addition to other features, the interior of the anterior part of the bladder and of the prostatic sheath. The posterior half, illustrated in figure 10, shows the peritoneal relations of the extra-peritoneal organs and the nature and extent of the areas denuded by the removal of the liver, stomach, mesentery, jejunum, ileum, colon, and transverse mesocolon.

The section was planned to pass anteriorly to the vena cava inferior in a plane that would cut symmetrical points upon the iliac crests some 2 inches from the anterior superior spines. The plane of section was then inclined posteriorly in order that it might traverse the central point of the perineum. The lower part of the section passes through the bladder, which was moderately distended and through the greatest transverse diameter of the prostate gland.

The part of the pancreas and a part of the duodenum that were removed in the original section from the posterior abdominal wall were replaced and fixed in situ prior to the removal of the mesocolon and mesentery. The anterior half of the bisected prostate was dissected from its sheath in order that the interior of the latter structure might be available for comparison with vagino-urethral sheath of Bellevue Model no. 3. Before the



Figs. 5 to 8 Bellevue Model no. 11, B, C, D, and the separately cast stomach, posterior view.

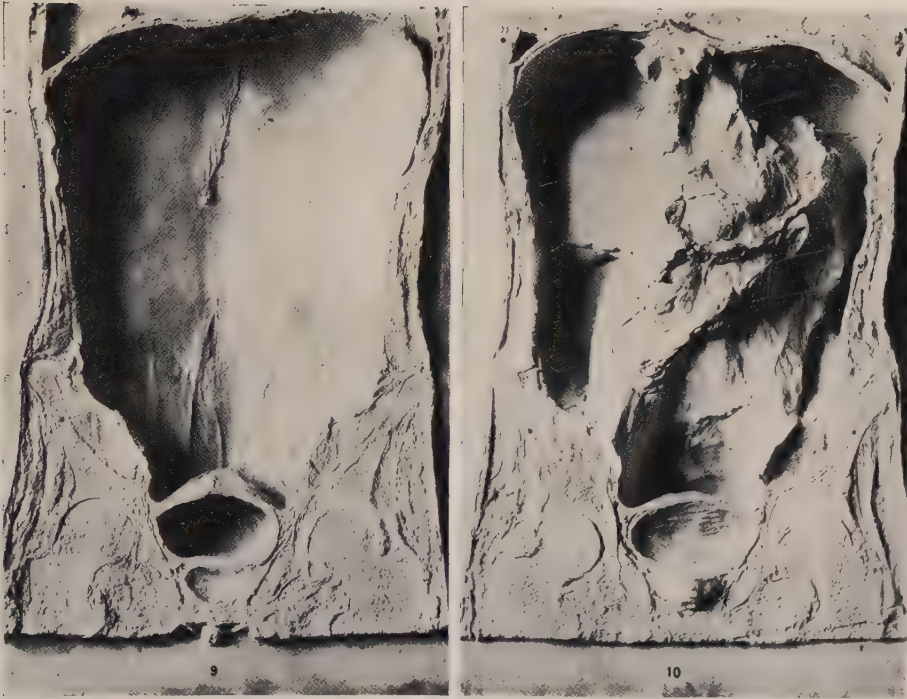
moulds were finally made, the anterior part of the bisected prostate was fixed in position to the posterior part of that organ and a redundant part of the psoas major muscle was also transferred from the anterior to the posterior part of the preparation.

The anterior surface of the right suprarenal of the subject used in the preparation of this model receives a complete covering from the peritoneum below the bare area of the liver. That part of the organ, therefore, has not its usual direct contact with the liver and the direct contact between the liver and the diaphragm is somewhat smaller than usual. A body, of which the external form closely resembles that of an inverted left suprarenal, although of somewhat larger size, lies in contact with the antero-medial margin of the right kidney.² Its lower pole, which is discernible in the cast illustrated in figure 9, comes into direct contact with the hepatic flexure of the colon.

As illustrating the reflections of the peritoneum from the abdominal and pelvic parietes, to the viscera, in the male subject, this model forms an excellent companion to Model II, B, C and D, which illustrates the reflections of the female peritoneum upon the viscera and from them to the abdominal wall. The peritoneal relations of the female pelvic viscera are illustrated in Bellevue Model no. III.³

² I am greatly indebted to Prof. Alexander Fraser, of this College, and to Prof. James Ewing, of Cornell Medical School, for their opinions as to the nature of this body. It is a fibroganglionic neuroma.

³ Spector, B., 1923. Reference models of the female pelvis. *Anat. Rec.*, vol. 25, no. 3, April, —.



Figs. 9 and 10 Bellevue Model no. IV; casts of the anterior and posterior parts of the interior of a male abdomen and pelvis from which the liver, stomach, jejunum, ileum, mesentery, colon, transverse mesocolon, etc., have been removed.

ON THE SMOOTH MUSCLE IN THE TURTLE'S HEART

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NINE FIGURES

While working out the comparative anatomy of the vertebrate heart musculature, I have been able to correct and extend the observations of Bottazzi ('07) and Laurens ('13) on the occurrence of smooth muscle in the heart of the turtle.

Laurens refers to the smooth-muscle cells: "They occur, as Bottazzi described them, as a layer immediately under the endothelium of the auricles, and are a continuation of the tunica media of the large veins. I have not been able to follow them into the ventricle" (p. 278).

I have found that the smooth muscle can be traced from the sinus venosus, through the atria into the ventricle, and thence out into the conus and great arteries of the heart.

A thin and interrupted layer can be found in the sinus venosus, where it is apparently a continuation of the muscle coat of the great veins. A far heavier coat lines the two atria and the atrial septum (figs. 1, 2, and 6). As the septum extends downward into the atrial canal, the smooth muscle gathers into two masses along its dorsal and ventral margins, and in the adjacent parts of the canal (fig. 3). Below the atrioventricular valves, the two masses of smooth muscle pass out into the ventricle. Each immediately breaks into many strands, which lie upon the cardiac trabeculae, just beneath the endocardium. The strands subdivide and radiate into the deepest recesses of the ventricular cavity (figs. 3, 4, and 5). At the root of the conus, muscle strands pass upwards into the muscular tunic of the great arteries (fig. 4).



Figs. 1 to 5 Sections through turtle heart. Van Gieson's stain. Fig. 1, frontal, others transverse sections. Fig. 2, through atrium; fig. 3, through atrial canal; figs. 4 and 5, through ventricle. Fig. 3, $\times 5\frac{1}{2}$; others, $\times 2\frac{1}{2}$. The heavy black represents smooth muscle. *A a v*, 'atrioventricular funnel' of cardiac muscle; *A d*, *A s*, right and left atrium; *B c*, bulbus cordis; *C a*, atrial canal; *C t*, connective tissue; *C v*, ventricular cavity; *m*, smooth muscle; *S a*, atrial septum; *S v*, sinus venosus; *V*, ventricle.

The atria have fairly complete smooth muscle coats. Within the ventricle the muscle fibers group into discrete radiating bundles, and form only a partial investment of the heart cavity.

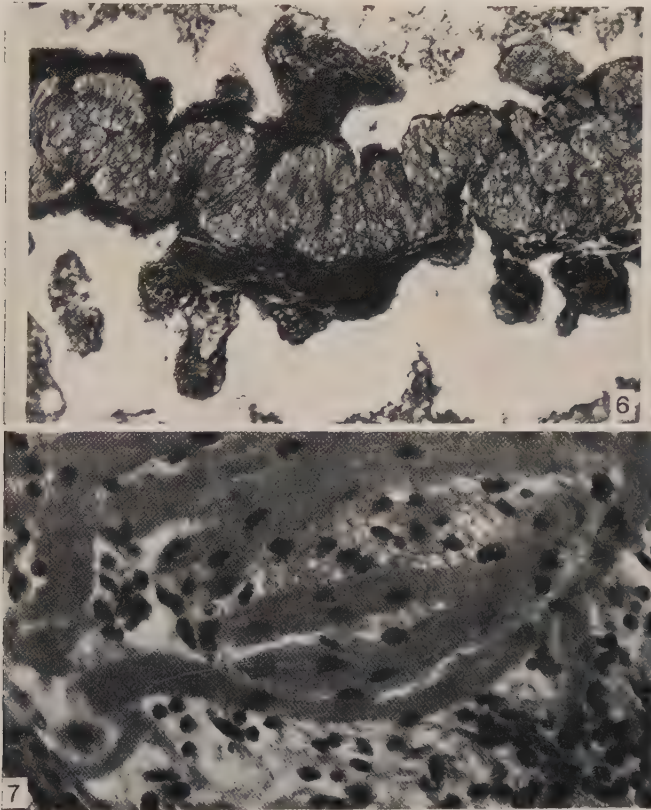


Fig. 6 Photomicrograph of cross-section of atrial septum. Mallory's connective-tissue stain. $\times 75$. The darker superficial tissue is smooth muscle.

Fig. 7 Photomicrograph of smooth muscle in atrium. Van Gieson's stain. $\times 340$.

In every case, however, the smooth muscle is found just beneath the endocardium.

In addition to such a peculiar distribution, the smooth muscle has several unusual histological features. The individual cells are embedded in a great sheath of collagen fiber which, with most

stains, completely hides everything but the nucleus. A typical field exhibits many corkscrew nuclei set in anastomosing strands of collagen fiber, which simulate branching protoplasm (fig. 7). The collagen reacts characteristically to the connective-tissue stains of Mallory, Ribbert, Unna, and Van Giesen.



Fig. 8 Camera-lucida drawing of smooth muscle in atrium. Aceto-phosphotungstic-acid hematoxylin. $\times 750$.

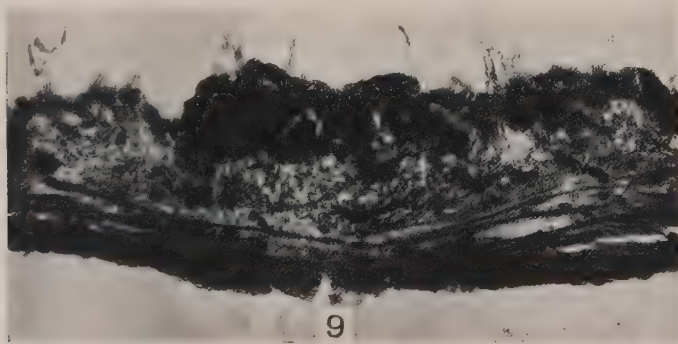


Fig. 9 Photomicrograph of wall of a branch of the mesenteric vein. Mallory's phosphotungstic-acid hematoxylin. $\times 260$. Note slender smooth-muscle fibers.

When, however, the tissue is stained with Mallory's phosphotungstic-acid hematoxylin, fine strands of true protoplasm appear within the collagen. The complete cells can be brought out with

a gold-chlorid impregnation, or by staining with very dilute aqueous gentian violet. A drastic, but more reliable method is to digest the sections a few minutes in strong acetic acid, then wash well and stain as usual with phosphotungstic-acid hematoxylin. The acetic acid breaks up the collagen enough to expose the muscle cells.

The cells, when brought out by any of these methods, are found to be exceedingly long, slender, and fusiform, with very long rod-shaped nuclei invariably thrown into corkscrew shape by contraction at fixation (fig. 8). The cells lie side by side, close to each other, but never joining. Each group is held together as a sort of contraction unit by the heavy sheath of collagen. Similar cells, not so obscured by collagen, can be found in the wall of any artery or vein (fig. 9).

It is hardly worth while to hazard a guess as to the function of smooth muscle in such a peculiar position. The cells may serve as an apparatus for tonic contraction. At any rate, we have here a second clear muscular connection between the atria and ventricle, in addition to the atrioventricular funnel of cardiac muscle.

NOTE.—The turtles on which this paper is based were obtained from an Indiana dealer. I am not certain, but I think they are Blanding's *Emys Blandingii* (Holbrook). I am indebted to Prof. N. B. Eddy, of the department of physiology, for the opinion that tonic contraction can well be the function of smooth muscle with such a distribution. On the other hand, Prof. F. T. Lewis has pointed out to me that smooth and cardiac muscle are closely akin developmentally, and that a conduction function for smooth muscle within the heart is not impossible.

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NOTE ON DEMONSTRATION OF MOTOR AND SENSORY NERVE-ENDINGS

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To those accustomed to using Ranvier's lemon-juice method for the demonstration of nerve-endings, it will be of interest to know that citric acid can be substituted for the lemon juice with equally good or even better results. The writer has found that excellent preparations of motor end plates and muscle spindles can be made in the following manner:

Intercostal muscles of the rabbit are removed in thin strips, freed from the rib, and placed in a 4 : 100 solution of citric acid in distilled water for twenty to thirty minutes in the dark. They are then rinsed in distilled water and transferred to a 1 : 100 solution of gold chloride in distilled water and kept in the dark for twenty to thirty minutes; then placed in a 33 per cent solution of formic acid (formic acid 33 cc., distilled water 67 cc.), kept in the dark for forty-eight hours, again rinsed in distilled water, and finally put into pure glycerin. After twenty-four to forty-eight hours the material may be examined under the microscope by taking small pieces and crushing them between two slides. In this way the nerve-endings are located and that area removed to a clean slide and mounted in glycerin under a cover-glass. The slide is then carefully cleaned close to the cover. Ring the cover with damar, balsam, paraffin, or cement. When not in use the slides should be kept in the dark.

Formic acid manufactured by J. T. Baker Chemical Company, Phillipsburg, New Jersey, has been found to be very satisfactory.

THE GROWTH AND VARIABILITY IN THE BODY WEIGHT OF THE NORWAY RAT (MUS NORVEGICUS)

HELEN DEAN KING

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THREE CHARTS

The investigations of Donaldson ('06, '09, '15), Slonaker ('12), Jackson ('13) and others have given a wealth of data regarding normal body growth in the domesticated albino rat. Information regarding the Norway rat, the wild prototype from which the Albinos were derived (Hatai, '07), is very meager, however, resting chiefly on the work of Miller ('11) and on scattered data noted by various investigators who were studying this species with the idea of exterminating it as a destructive pest and a menace to the health of mankind.

The present investigation was undertaken primarily for the purpose of obtaining norms for the Norway rat with which to compare the data for various series of hybrid and 'extracted' strains of rats. The growth and variability in the body weight of the first generation of Norways, born and reared in captivity, are described herein: subsequent papers will deal with litter production, the sex ratio, and the effects of domestication in this species.

MATERIAL AND METHOD

Wild Norway rats, trapped in various localities near Philadelphia, were brought into the laboratory in February and in March, 1919. These animals, comprising sixteen males and twenty females, were all at least three months of age when captured. In spite of confinement and changed conditions of environment and of nutrition, they remained in good health for many months, though only six of the females were known to

bear young. After twenty-one litters, containing a total of 139 young, were obtained, the parents were killed and dissected by Doctor Donaldson, and they were found to agree, in all essential respects, with the many wild Norways which he and Doctor Hatai had examined at various times during the past ten years.

Norway rats breed at all seasons of the year, as Lantz ('10) and Miller ('11) have noted, but the period of most pronounced sexual activity seems to be in the early spring. In captivity Norway females often devour their young at or soon after birth (Miller)—a tendency which these Norways exhibited in a marked degree. After a number of litters had been destroyed, it became apparent that rats could not be obtained for this study if the Norway females were depended upon to rear their young. The difficulty was overcome by removing the young Norways from the nest as soon as they were discovered and placing them with a lactating albino female. Norway young evidently have a very different odor from that of albino young, since in some cases the albino female objected to rearing the Norways and either destroyed or neglected them. If, however, the albino female was removed from the cage when the transfer of the young was made, and not replaced under half an hour, she usually accepted the foundlings and reared them as her own. A total of 110 offspring of wild Norways were reared, and many of them were kept until they were two years old. These animals, comprising 51 males and 59 females, form the material for the present study.

As it would seem to be an advantage in working with these Norways to have them tame, I made it a point to handle the young rats every day while they were with their foster mother. They submitted to this handling up to the time they were about thirty days of age, though they were very restless when held and usually tried to escape. After weaning they began to show increased resentment at handling and attempted to bite. The idea of taming them was abandoned, and subsequently they were slightly stupefied with ether when weighings were made.

The method used in obtaining the growth data for these Norways was the same as that employed in a former study of the growth and variability in the body weight of albino rats (King,

'15 a). The individuals were weighed separately at birth, if they were obtained before they had suckled. At thirteen and at thirty days of age the males and females of a litter were weighed collectively and the average weight for the two sexes recorded. After this time the rats were weighed separately, at intervals of thirty days, until they were two years old, when they were killed and the weight and condition of various organs determined.

The Norways lived under the same conditions of environment and of nutrition as the albino rats in our colony. It was necessary, however, to protect the cages with wire netting at all vulnerable points, as the rats would gnaw their way through a thick plank in a few hours. Although born in captivity, these Norways retained their inherent fear of man for many months. When the cage was approached the rats promptly went in hiding under the nesting material, and, if disturbed or the bedding removed, fear made them both reckless and savage, and they sprang at one's face if the cage door was opened. If they escaped from the cage they fought viciously when captured, and when returned to the cage they ran wildly about for some time. After several months they became more reconciled to captivity, and for the most part remained quietly in the corner of the cage when they were fed or the cage was cleaned.

GROWTH IN BODY WEIGHT OF THE NORWAY RAT

According to Miller ('11), Norway young weigh, on the average, 6.4 grams at birth, the males being slightly heavier than the females. In the four litters of this series that were obtained at the time of parturition the average weight of the twelve males was 5.34 grams; that of the fourteen females was 5.09 grams. The discrepancy between Miller's finding and mine may possibly be due to the fact that the Norways weighed by Miller had suckled before the weighings were made while those of my series were weighed immediately after birth.

Norway young seem to have a somewhat heavier birth weight than albino young, as a large number of determinations for Albinos show that the average birth weight of the males is 4.69 grams and that of the females is 4.5 grams (King, '15). It is

possible that the heavier birth weight of the Norway rats is due to the fact that in this form the gestation period is from $23\frac{1}{2}$ to $25\frac{1}{2}$ days (Miller), while in the albino strain the normal gestation is about $22\frac{1}{2}$ days. Growth of the albino fetus is very rapid during the later days of gestation, as Stotsenburg ('15) has shown, and if the Norway fetus grows at the same rate as the albino fetus the prolongation of the gestation period for even a single day might be expected to increase materially the birth weight of the young.

Although Norway females weigh less than the males at birth, they grow more actively during early life, as at thirteen and at thirty days of age their average body weight is greater than that of the males (table 1). After puberty (about ninety days) the males, as a rule, are considerably heavier than the females at all age periods for which records were taken. Albino rats show a weight relationship between the sexes much like that existing in the Norway strain (Donaldson, '06; Jackson, '13; King, '15 a).

Chart 1 shows graphs for the weight increase with age of the fifty-one males and fifty-nine females comprised in this series of Norways. The graph for the females runs slightly higher than that for the males until the sixty-day period when the graphs cross; beyond this point the graphs show increasing divergence with advancing age. Both graphs mount steadily upward until the 700-day period, thus indicating that the Norway rat tends to increase in body weight throughout adult life. There is a slight drop in the graphs at the end of the weighing period, but this can doubtless be attributed to the fact that many of the animals were suffering from so-called 'pneumonia,' as autopsies showed. During early life Norway rats are somewhat more resistant to 'pneumonia' than are albino rats, but they seem to lose this resistance after they are about eighteen months of age, and under laboratory conditions few of them can be kept free of this scourge in later life.

In order to compare the growth in body weight of Norway rats with that of Albinos, charts 2 and 3 have been constructed from the data given in table 1 of the present paper and from the data

in table 3 of a previous paper which show the growth of a series of stock albino rats comprising fifty males and sixty-seven females (King, '15 a). Both Norways and Albinos lived under similar housing conditions. The Albinos, however, were fed on a 'scrap' diet consisting of carefully sorted table refuse, while the Norways received the mixed ration (chiefly cooked cereals, meat, and fresh vegetables) which for sanitary reasons has replaced the former diet used in our colony. The two series contain approximately the same number of individuals and are probably fairly representative of the different stocks when reared under seemingly favorable conditions of environment and of nutrition.

Growth data collected by different investigators for various series of albino rats (Donaldson, '06; Slonaker, '12; Ferry, '13; Jackson, '13, et al.) all show that albino males grow very rapidly during the first 120 days of postnatal life, and that there is subsequently only a relatively slight increase in body weight. Males of the first generation of Norways born in captivity showed no marked acceleration in growth at any age period, as is indicated by the form of the graph in chart 2. Increase in body weight was steady and fairly uniform until the individuals were approaching senescence. In chart 2 the graph for the Norways crosses that for the Albinos at the 485-day period. No comparison can be made between the weight increase in the two forms at later age periods, as weight records for albino males have not been made, as yet, for any number of older animals.

Relative growth changes in body weight of Norway and albino females are similar to those for the males, as is shown by a comparison of the graphs in chart 3 with those in chart 2. In the case of the females, however, the graphs cross at an earlier age period (340 days), and subsequently the Norway females are heavier than the albino females at all age periods for which records are available (chart 3). Many of the Norway females are very heavy in later life, often weighing close to 400 grams—a weight that is rarely attained by any albino females.

The only observations, previously recorded, regarding the growth in body weight of Norway rats are those of Miller ('11) for a litter of eleven Norway young born in captivity. The

average body weight of these individuals, at thirteen days of age, sexes not separated, was 15.1 grams; at twenty-five days of age their average weight was 25.9 grams. These data are in accord with those given in the present paper.

TABLE 1

Data for eighteen litters of Norway rats, showing the increase in the weight of the body with age

AGE IN DAYS	MALES				FEMALES			
	Body weight in grams			No. indi- viduals	Body weight in grams			No. indi- viduals
	Average	Lowest	Highest		Average	Lowest	Highest	
13	15.4	12	21	51	16.3	12	21	59
30	33.4	26	41	51	37.0	27	45	59
60	81.9	49	144	51	75.2	47	110	59
90	115.9	68	201	51	100.5	70	152	59
120	148.6	70	245	51	122.6	77	184	59
151	176.1	87	284	51	142.0	98	201	56
182	195.9	110	300	51	153.5	111	256	54
212	218.4	128	330	51	172.1	117	272	50
243	235.4	140	374	50	189.3	133	278	52
273	253.5	154	379	50	204.2	131	327	51
304	265.5	156	380	50	209.8	123	301	47
334	276.7	170	395	50	221.5	128	345	49
365	287.3	184	428	48	232.8	152	344	47
395	297.4	185	442	48	242.2	135	383	47
425	312.2	200	457	47	247.5	156	380	43
455	322.3	188	471	47	260.5	172	359	44
486	332.0	226	459	46	257.9	182	382	43
516	335.0	226	476	43	264.1	163	373	37
547	345.6	231	486	41	261.5	173	375	36
578	358.0	233	516	39	266.5	168	360	34
608	361.8	231	518	38	271.9	184	339	31
639	369.9	236	540	37	279.7	190	367	30
670	374.5	240	546	37	284.4	210	355	26
700	364.2	243	518	34	283.4	200	376	25
730	362.1	231	505	27	278.9	197	341	19

It may be maintained that the slow growth of these Norways during the adolescent period is not normal for the species in its wild habitat, and that the form of the graphs shown in chart 1

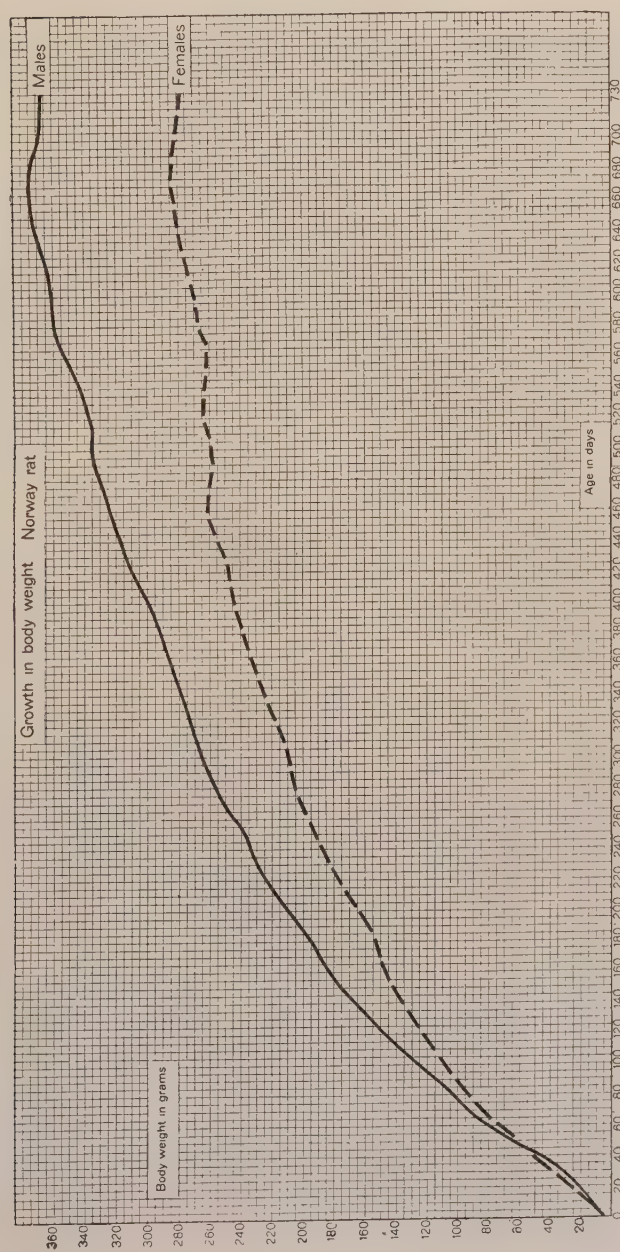


Chart 1 Graphs showing the growth in body weight of male and female Norway rats (data in table 1).

is the result of the conditions under which the animals lived. The rearing of the young Norway by albino foster mothers was not a factor that tended to retard early growth, as is shown by the following test that was made to determine this point. A litter of eight young born to a Norway female after she had been several months in the colony was separated into two groups of four each; one group was reared by the mother, the other by an albino female. At the thirteen-day period the young reared by the albino female weighed, on the average, 3 grams more than the young suckled by their mother. At thirty days of age there was an average difference of 4 grams in favor of the young reared by the albino female. During later life the individuals of the two groups showed no pronounced difference in body weight. Additional evidence that the suckling of the young by albino females did not retard the growth of these Norways is indicated by the fact that individuals in the second and third generations of Norways, that were all reared by their mothers, did not show the acceleration in growth during early life that is characteristic for the Albinos.

A second assumption that might be advanced to account for the slow growth of the young Norways is that the constant fear exhibited by the animals tended to inhibit their growth processes. Against such an assumption is the fact that some of the young Norways that were tamed by an assistant in the laboratory so that they showed no evidence of fear at handling did not grow at a more rapid rate than did the untamed animals.

It does not seem probable that the inherent growth tendency in the Norway rat would be greatly restricted by the environmental and nutritive conditions under which these rats lived, since these conditions would seem to be more favorable for growth than those under which the animals live in a wild state. The cages in which the Norways were confined were very large; food was varied and abundant, and the rats were not subjected to extremes of temperature nor harassed by enemies. These factors, it would seem, should serve to counterbalance any detrimental action of captivity on growth.

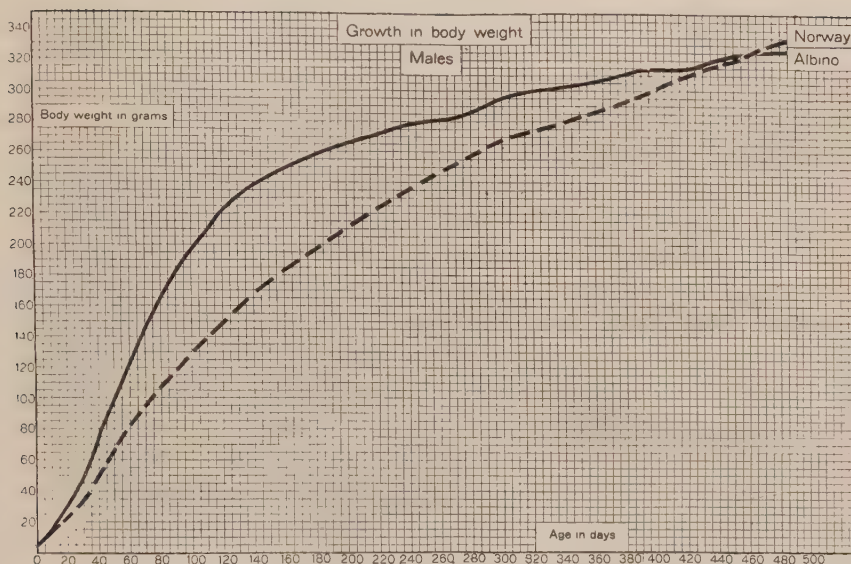


Chart 2 Graphs showing the growth in body weight of Norway and of stock albino males (data in table 1, and in table 3 of a previous paper; King, '15 a).

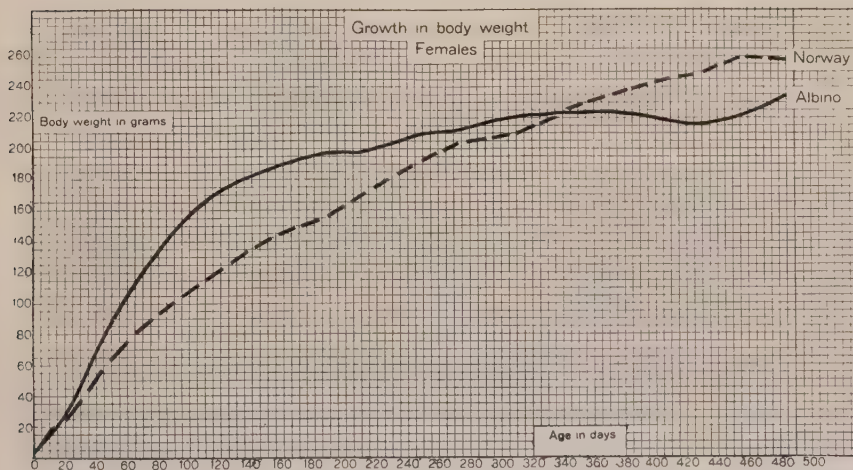


Chart 3 Graphs showing the growth in body weight of Norway and of stock albino females (data in table 1, and in table 3 of a previous paper; King, '15 a).

If the graphs in chart 2 and in chart 3 are fairly representative of the normal growth in body weight of Norway and of albino rats, then it is evident that growth energy is expended very gradually by the Norways and very rapidly by the Albinos. As rapid growth during early life seems to be characteristic of most domestic animals, it would appear that the acceleration in the growth of the Albinos is one of the effects of domestication on this form and that the Norways will tend to show the same acceleration in growth when they too become domesticated. In favor of this assumption is the fact that individuals in the later generations of these Norways grew somewhat more rapidly during early postnatal life than did those of the first generation. Graphs as plotted for animals in the second to the fifth generation are progressively more like those for the domesticated Albinos, and if the change in the form of the graphs continues, in a few more generations the Norways will show as great an acceleration of growth during adolescence as do the Albinos.

Not only in body growth, but also in other respects the Norways exhibit a slower development than do the Albinos. As Miller has stated, and as my observations show, Norway rats do not open their eyes before the sixteenth day of postnatal life (usually not until the beginning of the seventeenth day), while the eyes of albino rats are open when the animals are fifteen days old; in both forms the females tend to open their eyes a few hours before the males. Ping ('21) has found a relatively smaller number of large cells in the superior cervical sympathetic ganglion of the Norway rat at one day of age as compared with the number of such cells found in albino rats of like age—a fact which he interprets as indicating a retardation in the early development of these cells in the Norways.

VARIABILITY IN THE BODY WEIGHT OF NORWAY RATS

It is generally assumed that animals in a wild state are much more variable in many characters than are those under domestication, the variability being due, in great part, to environmental factors. Wild animals living in captivity under fairly uniform conditions of housing, of temperature and of nutrition might

therefore be assumed to lose much of the variability due to environmental causes and to exhibit chiefly that inherent in the species.

TABLE 2

Showing the coefficients of variation with their probable error for the body weights at different ages of a series of Norway rats (51 males and 59 females), and of a series of stock albino rats (50 males and 67 females)

AGE IN DAYS	NORWAY SERIES		ALBINO SERIES	
	Males	Females	Males	Females
13	12.2±0.82	14.6±0.90	11.8±0.79	11.4±0.76
30	13.0±0.86	17.3±1.07	10.2±0.68	11.0±0.74
60	24.1±1.60	19.8±1.22	17.0±1.14	15.7±1.05
90	26.2±1.74	16.9±1.04	14.8±0.99	12.5±0.95
120	28.2±1.88	16.0±0.99	13.4±0.90	10.3±0.75
151	24.2±1.61	16.6±1.03	13.3±0.89	10.4±0.73
182	23.4±1.55	17.8±1.13	14.2±1.22	12.3±0.90
212	21.8±1.45	17.9±1.20	14.0±0.96	12.4±0.91
243	23.7±1.60	18.2±1.26	13.9±0.99	12.6±0.91
273	22.4±1.50	19.1±1.27	13.4±0.99	11.5±0.89
304	21.5±1.45	19.9±1.38	14.0±1.11	10.3±0.79
334	19.8±1.33	20.6±1.40	13.7±1.13	10.8±0.87
365	19.5±1.33	20.4±1.41	13.0±1.16	10.7±0.91
395	19.5±1.34	22.3±1.54	12.6±1.22	11.5±0.98
425	17.6±1.22	21.1±1.47	13.4±1.32	10.9±0.94
455	19.2±1.32	18.9±1.35	13.6±1.67	8.9±0.99
486	17.3±1.21	19.5±1.41	15.0±2.06	13.4±1.77
516	17.4±1.26	18.3±1.43		
547	16.7±1.24	19.4±1.54		
578	18.3±1.39	16.4±1.34		
608	17.1±1.32	15.3±1.28		
639	17.4±1.36	15.8±1.37		
670	17.5±1.39	14.2±1.32		
700	17.7±1.36	11.9±1.14		
730	19.4±1.79	13.9±1.52		

The extent of variability in the body weights of these Norway rats was determined from a study of the coefficients of variation which were calculated for each age at which weighings were made. These coefficients, with their probable error, are shown in table 2, which also gives the coefficients of variation and probable error for the body weights of the series of stock Albinos with which the

growth of the Norways has been compared. The latter series of coefficients are reproduced from table 4 of a previous paper (King, '15 a).

Grouped data were used in calculating the coefficients of variation for the thirteen- and for the thirty-day periods in both Norways and Albinos, as only the average body weight for the rats of each sex in a given litter was recorded at these ages; individual data were used in obtaining the coefficients for all the other age periods noted.

In early postnatal life Norway females grow more rapidly than do the males and they are likewise more variable in body weight, judging from the relative size of the corresponding coefficients of variation (table 2). The variability in the body weight of the males has increased greatly by the time the animals are sixty days old, as is shown by the fact that the coefficient of variation for this period is nearly twice as large as that for the first period recorded. From this time until the rats are nearly a year old the males show a much more marked variability in body weight than do the females. Growth in body weight begins to slow down perceptibly in the females after 365 days of age (chart 1), yet from this time until the 547-day period the females are quite as variable in body weight as the males. The corresponding coefficients of variation for the two sexes covering this period show a difference in favor of the females, as a rule, yet these differences are, for the most part, within the limits of possible error and therefore are not significant. As old age approaches, variability in body weight decreases slightly in both sexes, but tends to be higher in the males than in the females.

In sixteen of the twenty-five age periods indicated in table 2 the coefficients of variation for the body weights of the males exceed those for the females; in eight of these cases the differences can be considered as significant, since they are more than three times the probable error. Where the coefficients for the females exceed those for the males the differences are too small to be of import. It would appear, therefore, that Norway males tend to be more variable in body weight than Norway females, especially during the period of early maturity. This finding agrees

with that for stock Albinos (Jackson, '13; King, '15 a), and accords with the view that males, in general, are more variable than females (Darwin, '71; Brooks, '83).

A comparison of the coefficients of variation for the Norways with corresponding coefficients for stock Albinos as given in the last two columns of table 2 shows that at all age periods Norway rats, both males and females, are much more variable in body weight than are albino rats. The maximum variability for both sexes of Albinos comes at sixty days; in the Norways the maximum variability comes at a much later period, 120 days for the males and 395 days for the females. In both forms the coefficients are relatively low for the first two age periods recorded; they rise to a maximum, and then decline somewhat as the animals approach senescence.

The results of this study support the view that variability is much greater in wild animals than in those under domestication. How much of this variability in the Norways is due to environmental factors and how much is inherent in the species remains to be determined. Although albinó rats have been living in a state of domestication for a long period, they show nevertheless an inherent tendency to variability in body weight at different age periods that is seemingly little affected by environmental conditions or by close inbreeding. Series of albino rats reared under fairly uniform conditions of housing and of nutrition and inbred brother and sister for twenty-five consecutive generations showed, at the end of this time, a variability in body weight at different age periods that was but little less than the variability exhibited by outbred stock Albinos (King, '18, '19). Whether the inherent tendency to variability in body weight is greater in the Norways than in the Albinos can be determined only after the Norways have become domesticated.

In man, according to Boas ('97) and Porter ('05), variability in body weight is correlated with rapidity of growth. Such a correlation is indicated for the albino rat also according to the data given in the last two columns of table 2, since here the coefficients of variation for body weight are highest for the age periods at which growth is most rapid (sixty to ninety days), and

then drop sharply when the period of rapid growth is at an end. According to my records, Norway rats grow very slowly during early postnatal life, and it is doubtless significant that the coefficients of variation for body weights at this age are relatively low for both sexes. The growth in the body weight of Norway rats continues at a fairly uniform rate for a long period of time. During this period the coefficients of variation for the females rise steadily, and they begin to fall only when the females are nearing the end of the reproductive period and their body weight has reached the level which it retains as long as the animals are in good condition. In the Norway males the correlation between rate of growth and variability in body weight is not as marked as in the case of the female. The coefficients of variation are very large during early maturity when the animals are growing vigorously: they remain at a high level during later adult life while the animals are still steadily increasing in body weight, and show no marked drop up to the time the animals are two years of age. In both Norways and Albinos there seems to be a correlation between variability in body weight and age, variability being relatively low during early postnatal life, increasing rapidly at puberty, and continuing at a high level until the end of the reproductive period, when it drops near the level of early life. Variability in body weight is seemingly correlated both with rate of growth and with age in the rat and in man. In this respect, as in many others (Donaldson, '06, '18), life processes in these two widely separated forms are in close accord.

SUMMARY

This paper gives the results of a study of the growth and variability in the body weight of eighteen litters of Norway rats, comprising fifty-one males and fifty-nine females, that were the offspring of wild Norways trapped in the vicinity of Philadelphia. The data given cover the period from birth until the animals were two years old.

Norway rats breed at all seasons of the year, but the period of most pronounced sexual activity is in the early spring.

Norway males have an average birth weight of 5.34 grams; females are slightly smaller than the males, having an average weight of 5.09 grams at birth.

During early postnatal life Norway females have an average body weight greater than that of the males. After sixty days of age the males, as a rule, are heavier than the females at all age periods.

Norway rats do not show the marked acceleration in body growth during early life that is characteristic of the albino strain. Increase in body weight progresses at a fairly uniform rate in both sexes until the animals are approaching senescence, the increase in the adult state being greater in the males than in the females (chart 1).

Albino rats are much heavier than Norway rats during adolescence and early maturity; in later life the body weights of the Norways exceed those of the Albinos (charts 1 and 2).

It seems probable that the slow growth of the Norways during early life is characteristic for the strain, and that the acceleration in the growth of the Albinos is one of the results of domestication.

Norway rats show a high degree of variability in body weight at all age periods (table 2). In early life the females are somewhat more variable than the males, but in the adult state the males tend to be the more variable.

Norway rats at all age periods are much more variable in body weight than are albino rats (table 2).

In the rat, as in man, variability in body weight is apparently correlated both with rapidity of growth and with age.

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REFERENCE MODELS OF THE FEMALE PELVIS

B. SPECTOR

Anatomical Laboratory, Bellevue Medical College, New York University

SEVEN FIGURES

Bellevue Model No. III consists of casts of five dissections of a female pelvis. It is the third of a series planned by Prof. H. D. Senior, and the dissections for it were executed, with the exception of that reproduced in cast D, figure 3, by Dr. S. Brock in 1917.

The subject which served for this model was twenty-two years old and had had one child. The date of parturition was not given in the hospital record, which states that she died of septicemia. Upon pelvic examination shortly before death, the uterus was found to be freely movable and there was no evidence of adnexal pathology. Prof. W. E. Studdiford noted that the pelvic floor was well formed, and suggested that it would make an excellent specimen for demonstration. After having injected the cadaver with 50 per cent formalin, the pelvic floor was dissected and cast (fig. 1, A). This cast proved so successful that it was decided to make others in order to show the arrangement of the viscera, the peritoneum, the fasciae, and the muscles of the pelvis. Therefore the viscera which were removed in order to make cast A were replaced and the whole pelvis divided in the midsagittal plane. Models B, C, and E were then cast from dissections of the right half and model D of the left half of the bisected pelvis.

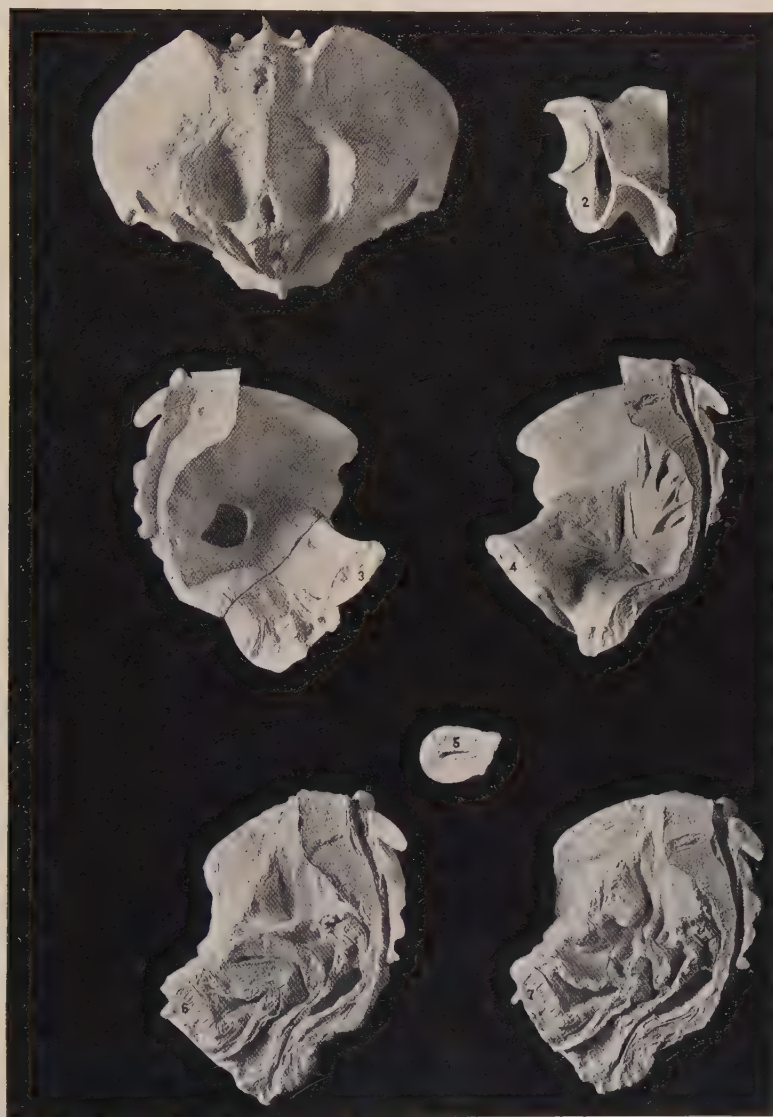
The features of each of the casts belonging to the series are as follows: In cast A, figure 1, the pelvic floor is dissected from both sides. The postero-inferior aspect of the right and left ischiorectal fossae and the antero-inferior aspect of the urogenital triangle is shown in figure 1. The internal pudendal artery, the

perineal nerve, and the dorsal nerve of the clitoris were dissected out of the canal on the lateral wall of the left ischio-rectal fossa. The right ischio- and bulbocavernosus muscles were removed and the crus clitoridis and vestibular bulb displayed. The irregularity at the left side of the vaginal orifice is probably the result of a tear which occurred during the time of labor; there is no evidence, however, that the tear involved the levator ani muscle.

The bladder and vagina were cut transversely and removed; the former at its base, the latter about an inch below its fornices. The large vascular area in this region, namely the pudendal, the vesical, and the uterovaginal plexuses of veins, and the common place of origin of the vesical, the rectovesical, and the rectal divisions of the visceral pelvic fascia are clearly in evidence.

Cast B, figure 6, is made from the right side of the bisected pelvis with the viscera and peritoneum in situ. The uterosacral ligament is recognized easily as a ridge running posterolateral from the cervix of the uterus to the sacrum. The round ligament comes well into view. The fundus of the uterus is about on a level with the upper margin of the symphysis pubis, the external os of the cervix on a level with the ischial spine and directed towards the posterior vaginal wall. While this topographical relationship of the uterus holds for the average case and is considered the usual one, the uterus itself appears somewhat sub-involuted. The fimbriated end of the uterine tube is prolapsed into the recto-uterine pouch—the result, in all probability, of a previous gonorrheal salpingitis, regarding which a definite history is obtainable. The plane of separation of the bladder wall from the anterior vaginal wall is well indicated.

Cast C, figure 7, also represents the right half of the bisected pelvis. It is similar to cast B except that the peritoneum was removed locally to display the following: the ovarian artery, the pampiniform plexus of veins, the suspensory ligament of the ovary, and the lower end of the right ureter. The uterus was cut transversely at the level of the internal os and removed; a separate cast, figure 5, was made of the body and fundus. Along the lateral aspect of the cervix, a thick mass of cellular tissue



Figures 1 to 7

can be seen; this surrounds the great vessels in this area and constitutes the parametrium. The uterine artery crosses the ureter obliquely, just before reaching the side of the uterus at the level of the internal os of the cervix.

Cast D, figure 3, demonstrates the general arrangement of the parietal pelvic fascia and the component layers of the visceral pelvic fascia, arising in common from the arcus tendineus, the thickened portion of the obturator fascia; it also shows definitely the area occupied by the pudendal, the vesical, and the uterovaginal plexuses of veins and the structures which pierce the pelvic diaphragm. This cast is really made up of two parts, an anterior and a posterior, made after the prepared specimen was cut through vertically from the left iliopectineal eminence to the middle of the ischial tuberosity.

Figure 2 is a cast of the anterior segment of the preparation viewed from behind and shows the obturator fascia, the obturator internus muscle having been removed, with two laminae arising from it; the superior lamina covers the upper surface of the levator ani muscle, while the inferior lamina covers its lower surface. The left vestibular bulb and the left ischio- and bulbocavernosus muscles also were removed to display the inferior layer of the urogenital diaphragm.

Cast E, figure 4, is made from the right half of the pelvis and illustrates the muscles which constitute the pelvic floor, devoid of their fascia and peritoneum. A very striking example is afforded of the manner in which the urogenital diaphragm bridges the interval that exists between the free anteromedial margins of the right and left levator ani muscles when casts D and E are placed together.

PROCEEDINGS OF THE AMERICAN ASSOCIATION
OF ANATOMISTS
THIRTY-NINTH SESSION

*Hull Laboratory of Anatomy, University of Chicago, Chicago,
Illinois, March 28, 29, and 30, 1923*

WEDNESDAY, MARCH 28, 1923

9:30 a.m. The Thirty-ninth Session of the American Association of Anatomists was called to order by President C. M. JACKSON, who appointed the following committees:

Committee on Nominations for 1923: Professor R. R. Bensley, Chairman, Professor G. Carl Huber and Professor H. H. Donaldson.

Auditing Committee: Professor H. E. Jordan, Chairman, and Professor R. M. Strong.

Professor George L. Streeter read the recommendations of the Journal Committee, to be presented at the business meeting of the Association.

The remainder of the morning session was devoted to the presentation of scientific papers.

THURSDAY, MARCH 29, 1923

12 noon. *Association Business Meeting.* The Secretary reported that the minutes for the Thirty-eighth Session were printed in full in the *Anatomical Record*, volume 23, no. 1, pp. 1-5, inclusive. On motion, seconded and carried, the minutes of the Thirty-eighth Session were approved by the Association as printed in the *Anatomical Record*.

Report of the Auditing Committee. Professor H. E. Jordan reported for the Auditing Committee as follows: "The undersigned Auditing Committee has examined the accounts of the Secretary-Treasurer from December 20, 1921, to December 31, 1922,¹ and has found them correct."

[Signed] H. E. JORDAN,
R. M. STRONG.

On motion the report of the Auditing Committee was accepted and adopted.

Report of the Treasurer. The Treasurer made the following report for the year 1922:

Balance on hand December 20, 1921, when accounts were last audited.....	\$ 172.86	
Receipts from dues of 1922.....	3,031.16	
Interest on deposits.....	14.53	
Total credits.....		\$3,218.55
Expenditures for 1922:		
Expenses, Secretary, New Haven Meeting	\$ 19.75	
Postage.....	20.00	
Printing and stationery.....	75.50	
Refund: overpayment of dues.....	.50	
Collection and exchange on drafts.....	1.20	
Wistar Institute, subscription for journals	2,840.50	
Total expenditures.....		2,957.45
Balance on hand, December 31, 1922.....		\$261.10
Balance on hand deposited in the name of the American Association of Anatomists in Alexander Brown & Sons, Baltimore, Md.		

On motion, the report of the Treasurer was accepted.

Election of Officers. The Secretary placed before the session the following nominations for office in the Association, as recommended by the Committee on Nominations, consisting of Professor A. S. Begg, Chairman, and Professors H. B. Ferris and C. H. Danforth:

For members of the Executive Committee with term expiring in 1926: Professor John Warren and Professor Richard E. Scammon.

On motion, seconded and carried, the Secretary was instructed to cast a ballot for the election of the above named.

New Members. The Secretary presented the following names recommended by the Executive Committee for election to membership in the Association:

- CHANG, CHUN, M.D., Professor of Anatomy, *Hopei University, Paotingfu, China.*
 FLORENCE, LAURA, B.Sc., M.A., Ph.D., Associate of the Rockefeller Institute for Medical Research, Department of Animal Pathology. *P. O. Box 284, Princeton, N. J.*

- GAMBLE, DEAN L., B.S., Ph.D., Assistant Professor in Zoölogy, *Cornell University, Ithaca, N. Y.*
- GOODRICH, EDWIN S., M.A., F.R.S., Professor of Zoölogy and Comparative Anatomy, *University of Oxford, Oxford, England.*
- HARRIS, HENRY A., M.B., B.S., M.R.C.S., L.R.C.P., Curator of the Museum of Anatomy, *University College, Gower Street, London W.C.1, England.*
- HIGGINS, GEORGE M., A.M., Ph.D., Assistant Professor of Zoölogy, *Knox College, Galesburg, Ill.*
- JOHNSTONE, PAUL N., A.B., Medical Student, *University of Georgia, Augusta, Ga.*
- KORNHAUSER, S. I., M.A., Ph.D., Professor of Anatomy, *University of Louisville, Medical School, Louisville, Ky.*
- LANGWORTHY, ORTHELLO R., A.B., A.M., M.D., Assistant in Anatomy, *Johns Hopkins Medical School, Baltimore, Md.*
- LEFHOLZ, ROTHWELL, A.B., A.M., Medical Student, *St. Louis University School of Medicine, St. Louis, Mo.*
- LOTH, EDWARD, M.D., Ph.D., Professor of Anatomy, *University of Warsaw, Warsaw, Poland.*
- MASTIN, EDWARD V., M.S., M.D., Instructor in Anatomy, *Washington University Medical School, St. Louis, Mo.*
- NADGIR, Y. G., M.D., Professor of Anatomy, *Grant Medical College, Bombay, India.*
- NOBLE, GLADWIN K., A.B., M.A., Ph.D., Curator of Herpetology, *American Museum of Natural History, New York City, N. Y.*
- TROTTER, MILDRED, A.B., M.D., Assistant in Anatomy, *Washington University Medical College, St. Louis, Mo.*
- WINDLE, WILLIAM F., B.S., Assistant in Anatomy, *Northwestern University Medical School, Chicago, Ill.*

On motion, seconded and carried, all candidates proposed by the Executive Committee were declared duly elected.

Dropped Members. The Secretary then announced the following names as dropped from the list of members on account of non-payment of dues for the past two years:

- Dr. CHARLES M. BYRNES, Johns Hopkins Hospital, Baltimore, Md.
- Dr. JAMES R. CASH, Johns Hopkins Hospital, Baltimore, Md.
- Dr. DAVID M. DAVIS, Johns Hopkins Hospital, Baltimore, Md.
- Dr. JEAN FIRKET, University of Liege, Liege, Belgium.
- Dr. C. PING, Government Teachers College, Nanking, China.
- Dr. JOHN PRACHER, St. Mary's Hospital, Evansville, Ind.
- Dr. HARRY W. VANCE, Johns Hopkins Hospital, Baltimore, Md.

Resignations of Members. The Secretary reported that the following members had resigned from the Association during 1922:

- Dr. WILMER BAKER, Tulane University, New Orleans, La.
- Dr. CLAUDE S. BECK, Johns Hopkins Medical School, Baltimore, Md.
- Dr. DAVID CHEEVER, Harvard Medical School, Boston, Mass.

Dr. WILLIAM O. OWEN, United States Army Medical Corps, Washington, D. C.
Dr. FERDINAND SCHMITTER, United States Army Medical Corps, Albany, N. Y.

Death of Members. The Secretary then announced, on behalf of the Executive Committee, that during the past year the Association had suffered great loss in the death of the following members:

Dr. FRANCIS C. FORD, Hahnemann Medical College, Chicago, Ill.
Dr. W. S. HALSTED, Johns Hopkins Hospital, Baltimore, Md.
Dr. EDWARD L. MELLUS, Brookline, Mass.
Dr. J. M. STOTSENBURG, Wistar Institute, Philadelphia, Pa.
Dr. HARRY O. WHITE, Los Angeles, Calif.

Place of Next Meeting. It was announced that the Executive Committee had voted to hold the next annual meeting at Easter, 1924, in some mid-western city, the exact place to be determined later. If possible, a joint session with the American Association of Pathologists and Bacteriologists will be arranged.

Member of Journal Committee. The Executive Committee nominated the following members of the Association to serve on the Journal Committee as organized at the Thirty-seventh Session of the Association: Professor Irving Hardesty, Professor T. Wingate Todd.

Professor C. R. Bardeen was nominated from the floor.

As a result of the election, Professor C. R. Bardeen was chosen to serve for five years.

Address by Professor Huntington. The Secretary read before the Association a telegram announcing that Professor George S. Huntington was prevented from delivering the formal address at the third morning session of the Association. On motion, the Secretary was instructed to send a message to Professor Huntington expressing the profound regret of the Association at his absence from the meetings.

Union of American Biological Societies. The Secretary reported that the American Association of Anatomists had been invited to become a member of the Union of American Biological Societies. The Executive Committee recommended that the Association join this newly constituted Union. Professor F. R. Lillie spoke briefly of the purposes and organization of the

Union and of the desirability of all biological societies entering into such a federation.

It was moved, seconded, and carried that the American Association of Anatomists join the Union of American Biological Societies.

It was further moved, seconded, and carried that the President of the Association be authorized to appoint two members to serve as representatives of the Association of Anatomists on the Council of the Union of American Biological Societies, the period of service to terminate at the next annual meeting. The President made the following appointments for this purpose: Professor H. H. Donaldson and Professor L. H. Weed.

Report of the Journal Committee. Professor G. L. Streeter presented the following recommendations on behalf of the Journal Committee:

1. That the present arrangement with The Wistar Institute for supplying the members of the Association with the several Journals in return for dues be discontinued and that the offer of The Wistar Institute to supply the Journals to the members at a discount of at least 25 per cent from the regular subscription rates be accepted.

It is understood that in exchange for the dues of the present year the members will receive from January 1, 1923, the Journals subscribed for until in each case the equivalent of one volume has been received.

2. That the constitution of the Association be amended so that the first sentence of Article VI be made to read as follows:

"The annual dues of the Association shall be determined at each annual meeting for the ensuing year."

3. That beginning with the next annual meeting the abstracts be printed as a number of the Anatomical Record preceding the meeting and that the proceedings of the meeting be printed in a subsequent number.

[Signed] C. M. JACKSON,
C. R. BARDEEN,
H. M. EVANS,
G. L. STREETER, *Chairman.*

It was moved and seconded that the recommendations of the Committee be accepted by the Association. After discussion, the motion was carried.

On motion, the business session then adjourned.

Dr. WILLIAM O. OWEN, United States Army Medical Corps, Washington, D. C.
Dr. FERDINAND SCHMITTER, United States Army Medical Corps, Albany, N. Y.

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[Signed] C. M. JACKSON,
C. R. BARDEEN,
H. M. EVANS,
G. L. STREETER, *Chairman.*

It was moved and seconded that the recommendations of the Committee be accepted by the Association. After discussion, the motion was carried.

On motion, the business session then adjourned.

FRIDAY, MARCH 30, 1923

12:30 p.m. A short business meeting followed the morning scientific session, with Vice-President Harold D. Senior presiding.

Professor H. H. Donaldson presented the following resolution:

RESOLVED: That the American Association of Anatomists desires to express to the University of Chicago and to the Department of Anatomy its appreciation of the many courtesies extended to the members of the Association during the present meeting.

Moved, seconded, and unanimously voted.

On motion, the Session adjourned.

LEWIS H. WEED,

Secretary of the Thirty-ninth Session of the
American Association of Anatomists.

ABSTRACTS

PAPERS READ BY TITLE

1. *Regulatory adjustment in the arteries of a dicephalous embryo.* MABEL BISHOP, Department of Anatomy, University of Chicago. (Introduced by C. Judson Herrick.)

Report of a study of the heart and head arteries in a symmetrical pig embryo of 22 mm.

The heart is normal. Right and left common carotids pass upward, the former conveying blood to the right head (A), the latter to the left head (B), with only slight variations, viz., the right external carotid of head B supplies the right half of the tongue of its own head and the left half of the tongue of head A. It is therefore a median external carotid artery, and after giving off these lingual branches continues in the midplane as a median (conjoined) facial artery, distributing branches to the structures normally supplied by a facial artery.

The common carotids become right and left internal carotids as normally expected, one to head A the other to head B. In the region of the hypophysis of each head each internal carotid enters into the anlage of the circle of Willis characteristic of normal embryos. One adjustment is noticeable, however. The internal ear cartilages are conjoined structures and the left internal carotid of head A gives origin to a median stapedia artery which passes through the conjoined stapedia cartilages.

These regulatory adjustments of the head arteries confirm a previous study of the regulatory adjustments of the nervous system, for in both the embryological and morphological relations are clearly demonstrable, and wherever normal relations are possible, or near possible, they assert themselves in convincing manner.

2. *The incidence of the supracondyloid process in the insane.* LEE D. CADY, Washington University. (Introduced by R. J. Terry.)

One thousand insane white subjects were examined by digital palpation of the arm bones, and twelve individuals were found to have supracondyloid processes. In 500 men only one process was found; in 500 women twelve processes. The presence of all the processes was confirmed by x-ray examination. There is no significant difference in these figures and those of Terry's found in white dispensary patients. The high incidence of Nicolas (5.2 per cent in 115 insane individuals) is not borne out by this investigation.

3. *The growth of the vertebral column in the human fetus.* L. A. CALKINS, Department of Anatomy, University of Minnesota.

Graphical representation of measurements of the vertebral column in human fetuses, from three months to term, results in straight line curves. These curves demonstrate a constant relation of the column and its various segments and parts to the standing height, and to one another, throughout the fetal period. Empirical formulae for these straight lines verify the law of cephalocaudal development, very exactly. Even the growth rates of the individual vertebrae in both the embryonic and fetal periods demonstrate this principle.

4. *What valves are functional in the heart of pig embryos of 9 to 15 mm.?* C. CHANG, Hopei University. (Introduced by F. T. Lewis.)

In the Harvard Laboratory, Doctor Lewis expressed to me his belief that the only functional valve in the hearts of 12 mm. pigs is that of the sinus venosus. He showed me a case of 'insufficiency' or forcing of that valve, and suggested the study here provisionally reported. Ink introduced into right or left ventricles readily flows through the atrioventricular openings into the atria, showing that these orifices are without effective valves. Sometimes slight resistance is encountered in filling the aorta, as if the semilunar valves; before their cusps become cups, are obstructions, hindering outflow as well as inflow. A greater pressure, though still slight (approximately 2 mm. of mercury), is required to force the valve of the sinus venosus. Its right flap proves more rigid than the left. Three abnormal valves have been modeled, showing partial inversion of flaps, or a subdivision of the right one, emphasizing the possibility of 'insufficiency of the valve of the sinus venosus.' The pulmonary vein may be filled by injecting either right or left ventricle, in cases in which ink has not traversed the pulmonary artery, thus suggesting that its blood may, at the outset, flow from the heart as well as towards it; this, however, requires more critical study. If, at this stage, the atrioventricular orifices close in systole, it is not by the approximation of flaps, but by the constriction of thick-walled channels, which, however, seems not to occur, at least in the right side of the heart.

5. *Changes in the vascularity of the brain between birth and maturity.* E. HORNE CRAIGIE, Department of Biology, University of Toronto.

An investigation of the relative vascularity of selected parts of the brain stem and cerebellum of the albino rat at various ages between birth and maturity is in progress. Results so far obtained indicate that the most important changes take place prior to the age of twenty days. At twenty days, the average vascularity of the fourteen centers studied is somewhat greater than that found in the adult, but the general relations between the parts in respect of capillary richness are similar. At birth, however, the average vascularity of the fourteen centers is much less, and there is also much less difference between these centers. The greater capillary richness of the sensory nuclei as compared with the motor ones, which is found in the adult, no longer appears, and even the fasciculus longitudinalis medialis, representing the white matter, is about as richly supplied as is much of the gray matter.

6. *Polychromasia in relation to sexual maturity.* V. E. EMMEL and C. O. MILLER, Department of Anatomy, University of Illinois.

In a previous report on polychromasia in relation to gestation and lactation (Anat. Rec., vol. 23, p. 17, 1922) evidence was advanced showing in male rats a gradual decline in the percentage of polychromasia from 21.3 per cent at birth to 5.1 per cent on the fifty-sixth day. This erythrocytic behavior was discussed as suggestive of a possible correlation between postnatal reduction in polychromasia and the attainment of sexual maturity. The results of the present study of a series of female rats demonstrates a decrease in polychromasia from an average of 28.8 per cent at birth to 3.8 per cent at the onset of the first oestrous cycle, the latter percentage indicating the attainment of a level of polychromasia approximately equivalent to adult conditions.

7. *Stability of hormones in the anterior hypophysis.* C. F. FLOWER, C. E. FÖRKNER, W. E. KELLUM, A. T. WALKER, P. E. SMITH, and H. M. EVANS, Anatomical Laboratory, University of California.

We have endeavored to see whether characteristic effects of anterior hypophyseal fluid on growth and ovulation were destroyed by oxidation or by other influences within a short time. The standard preparation used in our experimental work has been submitted for one hour to bubbling oxygen; other lots of the standard preparation have been protected against bacterial or autolytic destruction by 8 per cent ethyl alcohol and left at room temperature for one week. Neither of these methods of treatment has successfully destroyed the two highly characteristic effects of this substance.

8. *Separation of the principle in the anterior hypophysis affecting ovulation from that controlling general body growth.* C. F. FLOWER, C. E. FÖRKNER, W. E. KELLUM, A. T. WALKER, P. E. SMITH, and H. M. EVANS, Anatomical Laboratory, University of California.

It has been demonstrated through the work of Evans and Long that parenteral administration to rats of the bovine anterior hypophysis causes a syndrome characterized by body overgrowth, or gigantism, and by repression of ovulation due to lutein cell transformation of ovarian follicles. Alcohol has been carefully added to our standard aqueous extract and suspension of anterior hypophyseal substance so that the concentration of alcohol reached was 50 per cent and the precipitated proteins were separated by centrifugation. Both the precipitated material and the supernatant fluid have been administered intraperitoneally to rats in doses identical with control doses of the unaltered standard preparation. The supernatant fluid still possessed the principle, or principles, affecting the gonads since ovulation (as determined by the recurrence of typical vaginal changes) was prevented, and sections of the ovaries show the same histological picture as in animals submitted to treatment with unaltered fluid from the whole anterior-lobe substance. Such animals, however, were not accelerated in growth, the principle conveying this impulse having evidently been destroyed or carried down with the protein precipitation.

9. *Independent behavior of monocytes in experimental leucocytosis.* CLAUDE E. FÖRKNER and WILBUR E. KELLUM, Anatomical Laboratory, University of California. (Introduced by H. M. Evans.)

The vital staining method for the recognition of monocytes makes it possible to trace these cells in experimental leucocytosis. Experimental leucocytoses were produced in rabbits by submitting them to exposure to the x-ray and to heat, more especially since these agents have been reported as exercising a selective effect upon the lymphoid cell. In only a portion of the experiments were the leucocytoses which were constantly produced not participated in by both myeloid and lymphoid cells. The monocytes participated in these oscillations only very slightly. In some cases they appeared to follow the lymphoid cells in their slight fluctuations, while in others their response more nearly paralleled that of the myeloid cells. Were they related to either cell strain we should expect in them accurately corresponding oscillations. Their fluctuations were determined by the same agents producing

fluctuations in all the blood cells, but could only be deemed independent of those shown by the other cells

10. *Participation of the mammary gland in the changes of pseudopregnancy in the rat.*

MARY E. FREYER and HERBERT M. EVANS, Anatomical Laboratory, University of California.

In rats in which the condition of 'pseudopregnancy' has been produced either by congress with vasectomized males or by mechanical stimulation of the cervical canal, the mammary epithelial tree participates in the changes. In pseudopregnancy, verified by the detection of a surface columnar vaginal epithelium, the mammary tree when spread, strained and mounted in toto is seen to form a characteristic picture never found during the normal cycle and resembling, though seldom equaling, its condition at about the eleventh day of normal pregnancy. The picture consists in the multiplicity of small twig-like buds and the beginning production of more expanded, true alveolar clusters. The changes have been typically produced in two instances in which the oestrous cycle was characteristically prolonged by mating with a vasectomized male, but in which vaginal changes were absent.

11. *On excretion of nitrogenous waste as a function of the contractile vacuole.*

RUTH B. HOWLAND, Cornell University Medical College.

Observations made on the fluid of the contractile vacuole of certain protozoa subsequent to the application of the murexide test give no evidence of the presence of uric acid. This result is not in accord with the generally accepted conclusion of Griffiths ('89). Mass cultures of *Amoeba verrucosa*, killed in 50 per cent alcohol, show the contractile vacuoles fixed at partial or complete diastole in a large percentage of cases. Treatment with nitric acid and ammonia in the manner described by Griffiths fails to induce the purple color characteristic of ammonium purpurate. Dark-field examination of the vacuoles shows the fluid to be structureless. Concentrated cultures of *Paramoecium caudatum* subjected to the same test also give a negative result. This would indicate, therefore, either that uric acid is not an end product of the katabolic activity of these protozoa, or that the murexide test is not sufficiently sensitive to give a satisfactory reaction in this case. In view of the latter possibility, other, and more specific, methods for determining the presence of uric acid in very minute quantities are being applied at the present time.

12. *The comparative morphology of the corpus striatum.*

J. B. JOHNSTON, University of Minnesota.

The corpus striatum consists of two parts from the standpoint of time of appearance in the vertebrate series and of three parts from the standpoint of function. The three parts are: the nucleus lentiformis, the bed of the stria terminalis, and the nucleus caudatus. The first two of these are recognized in fishes and constitute the old or basic part of the corpus striatum—literally a palaeostriatum.

The stria-bed includes part of what is known in man as the head of the caudate nucleus, is continuous with the nucleus accumbens and the bed of the anterior commissure, extends without interruption to the temporal pole and fuses there with the central and medial nuclei of the amygdaloid complex. This stria-bed is essentially the ventricular layer of the lateral olfactory area of all vertebrates. The stria terminalis includes bundles of fibers connecting the rostromedial with the

caudolateral olfactory areas, and bundles connecting the caudolateral area (amygdala) with lower centers.

The nucleus lentiformis is derived from the primitive somatic correlation tissue in the forebrain of fishes.

The nucleus caudatus (excluding that part assigned to the stria-bed) is derived from the lateral border of the general pallium and represents the first step in the expansion of pallial area by folding. The hypertrophy forms an internal ridge which appears first in reptiles. This is therefore literally a neostriatum. The nucleus caudatus and the stria-bed appear in embryonic and foetal human brains as two prominent parallel ridges in the floor of the lateral ventricle.

13. *The evolution of the amygdaloid complex.* J. B. JOHNSTON, University of Minnesota.

As the hemisphere in vertebrates elongated the lateral olfactory area continued into the temporal pole accompanied by the lateral olfactory tract. The lateral border of the area which meets the general pallium came to serve as an olfactosomatic correlating center. The medial border retained more primitive olfactory functions and maintained its close connection with the tuberculum olfactorium and the ventricular layer which came to imbed the stria terminalis. The caudal end of this medial border is known as the nucleus of the lateral olfactory tract.

When animals emerged from the water to live on land the manifold stimuli which they experienced gave a vastly increased importance to the olfactosomatic correlation. There resulted in reptiles and mammals an hypertrophy of the temporal portion of the lateral olfactory area (area pyriformis) which brought about an immigration of cells and infolding of the wall along the line of the lateral olfactory tract. The sulcus endorhinalis was thus converted in its temporal part into a fissura amygdalae. This was the first step in the expansion of the olfactory pallium by folding, closely comparable with the case of the nucleus caudatus and general pallium.

The amygdaloid complex consists of certain nuclei, the central, medial and cortical, which represent the primitive lateral olfactory area and are closely connected with the bed of the stria terminalis; and of other nuclei, the basal and lateral, which are products of the infolding at the amygdaloid fissure and are more intimately concerned with olfactosomatic correlation.

14. *The normal blood picture in the rabbit by the vital-staining method.* WILBUR E. KELLUM and CLAUDE E. FORKNER, Anatomical Laboratory, University of California. (Introduced by H. M. Evans.)

During the past few months we have had occasion to study the normal blood picture of this important laboratory animal by the method of vital staining developed in this laboratory (Simpson, M. E., Anat. Rec., April, 1921). This method is of importance inasmuch as it is the only method which provides a satisfactory means of differentiating the monocytes from the other mononuclear leucocytes. The following average counts are taken from 160 counts upon eight normal, infection-free rabbits. Amphophiles 37.9 per cent, basophiles 7.8 per cent, eosinophiles 1.7 per cent, monocytes 6.2 per cent, lymphocytes 46.5 per cent; total 7920. We observed that while certain individuals showed wide variations in the blood picture from day to day, in the majority of cases the picture is comparatively constant. The following

is an example of the fluctuations in one animal over a period of about a month: Amphophiles 22 to 32 per cent, basophiles 3 to 8 per cent, eosinophiles 1 to 4 per cent, monocytes 4 to 8 per cent, lymphocytes 50 to 60 per cent; total 3900 to 4500. It is important that in making such counts a uniform hyperemia be induced in the ear by a warm water bath immediately before taking the blood.

15. *On the deep lymphatic vessels of the liver.* F. C. LEE, Department of Anatomy, The Johns Hopkins Medical School.

A retrograde injection of the lymphatic vessels of the liver in a cat was secured by first ligating the thoracic duct in the thoracic cavity, then injecting pilocarpine into the abdominal cavity to increase the pressure on the lymphatic system because of plain muscle contraction, and finally injecting the mesenteric lymphatic vessels in a centripetal direction. The injection mass entered the liver and filled the lymphatic vessels near the hilum.

Some of the liver tissue was cleared in oil of wintergreen, and the bulk of the remaining tissue was imbedded in celloidin and cut serially. From this material it was seen that the lymph vessels formed a large network in Glisson's capsule surrounding chiefly the portal vein, but also the hepatic artery and bile ducts. This lymphatic plexus formed numerous anastomoses and enmeshed the liver lobules. All the finer lymph radicles were definitely perilobular, and did not enter the lobule. From the periportal plexus lymph vessels were given off directly to the vessels in the wall of the large hepatic veins. These communications were spacious where a large hepatic vein was in close proximity to a large portal vein; but they were delicate peripherally where the relation was between small hepatic veins and small portal veins. Thus the lymph of the liver may leave at the hilum or along the hepatic veins.

16. *Mitochondria in the visual cells of the fowl.* MARGARET REED LEWIS, Carnegie Institution of Washington.

Since the question of the part taken by the mitochondria in the development of the visual cells is still undecided, it seemed of moment to see what staining of the living visual cells with janus green would contribute to this question. In the living cells certain granules, which stain with janus green, were present throughout the development of these cells, even in the rod and cone cells of the adult fowl. There was, however, no trace of janus green in those structures which in fixed material stain more or less like mitochondria.

In the adult visual cells the janus-green granules were collected into a mass just proximal to the fat-globule. In those without fat-globules the janus-green granules were located in the distal end of the inner segment. This mass of janus-green granules corresponds to what has been described as the ellipsoid in fixed and stained material. There were also a few scattered stained granules around the nucleus. The visual cells of just-hatched chicks contained janus-green granules in the same positions as the adult cells. In cells of progressively younger embryos the stained granules were less aggregated until in embryos of fifteen days' incubation the janus-green granules were mostly filaments scattered throughout the elongated portion of the cell and arranged parallel to the long axis of the cell. There were also more granules around the nucleus. In embryos of eleven days' incubation the janus-green granules were already largely segregated into the protoplasmic bud.

The filamentous outer segment of the visual cells exhibited changes similar to those of the sarcostyles of the living wing muscle of the house-fly. They contracted, became cross-striated, segmented, fragmented, or broken up into discs.

17. *The transformation of mesenchyme into mesothelium in tissue cultures.* WARREN H. LEWIS, Carnegie Institution of Washington.

The reticular multipolar mesenchyme cells which migrate out onto the cover-glass in most cultures of the embryonic chick heart form a loose adherent reticulum that may change, under the eye, into a flat mesothelial membrane. This is accomplished by a partial withdrawal of the long processes and a spreading out of the cytoplasm of each cell until it abuts the cytoplasm of its neighbors in fairly sharp lines, straight and curved. These sharp intercellular boundaries can be seen in the living cultures as well as often iodine fixation or silver-nitrate treatment. There is often some overlapping of cell edges as well. Thus, next the cover-glass the edges of two cells may abut sharply, while deeper focusing in the same region shows overlapping. At the periphery of the membrane transition cells between mesenchyme and mesothelium are present. As the cultures get older the mesothelial membranes may loosen up into reticular cells which finally contract into more or less globular forms. The mesothelial cells often show on fixation quite marked fibrillae extending to the periphery of the cell. These appear to be coagulation fibrils similar to those seen in fixed endothelial cells in cultures of the liver.

18. *Cultivation of heart muscle from chick embryos (four to eleven days old) in Locke-bouillon-dextrose medium.* WARREN H. LEWIS, Carnegie Institute of Washington.

In about 8 per cent of the cultures muscle cells migrate out along the cover-glass. These cells form either an adherent reticulum (not a syncytium) or an adherent membrane in which the cells are stuck together. Where the reticulum is thick it is not possible to determine the relations of the cells to one another, but at the periphery and in the membranes distinct outlines can often be followed. Single cells may become entirely isolated. Individual cells within the outgrowth may pulsate at rates different from those of the explant, reticulum, or membrane, or even from adherent neighboring cells, indicating physiological as well as morphological independence. Myo-fibrils, not to be seen in the living cells, appear on fixation. They vary in length and thickness down to the limits of visibility and are probably artifacts dependent upon the manner in which the contractile substance coagulates. Some fixed cells show cross-striated fibrillae, some only smooth ones, and some none at all; all such cells may beat rhythmically. Heart muscle undergoes retrogressive changes in the explant or as it migrates out, as indicated by the condition of the fixed contractile substance. Myofibrillae do not extend from one cell to another. Heart muscle in the living animal, both embryo and adult, is probably likewise composed of individual cells without fibrillae. These questions cannot be settled from ordinary fixed and sectioned material. Glycogen, frequently present in the muscle, was not observed in any other types of cells in these cultures.

19. *Changes in the weights of the various organs and parts of the leopard frog (*Rana pipiens*) at different stages of inanition.* M. D. OTT, Institute of Anatomy, University of Minnesota.

Twenty controls of each sex were autopsied in the fall, with smaller groups during

hibernation and subsequent stages of inanition at losses of 10 to 60 per cent in body weight.

During hibernation the body weight remained nearly stationary or increased slightly, due chiefly to accumulation of water in the lymph spaces of the trunk. The trunk, lungs, and 'remainder' increase in weight during this period; the musculature, spleen, stomach, intestines, fat-bodies, and kidneys decrease, while the other parts change but slightly.

During subsequent stages of inanition, the following lose relatively less than the whole body: lungs (increase), eyeballs (increase), head, skeleton, brain, spinal cord, tongue, heart (female), stomach-intestines, 'remainder,' and spleen (variable).

With relative loss approximately equal to that of the body (difference less than 10 per cent) are: limbs, integument, musculature (female), heart (male), and kidneys.

With relative loss greater than that of the body are: trunk, musculature (male), liver, and especially the fat-bodies and sexual organs.

There is a sex difference in normal body weight, the female being heavier on account of the ovaries and oviducts. The resorption of the ova apparently spares certain organs of the female during the later stages of inanition. Spawning and copulation are inhibited.

In percentage of dry substance most organs show but little change during hibernation, with decrease during subsequent inanition, especially in the later stages (some sex differences). The integument, brain, spinal cord, eyeballs, heart, spleen, kidneys, and testes remain nearly stationary; liver (female) increases.

20. *On the incidence of the supracondyloid process.* IRENE PIEPER, Washington University Medical School. (Introduced by R. J. Terry.)

This work was undertaken to determine the frequency of a certain variation in a group selected on the basis of physical soundness and intelligence as compared with its occurrence in groups made up, *a*) of the undernourished and mentally lower grades and, *b*) of mental defectives. Material for the sound group was sought in the various departments of a university; that of group *a* had been derived from a public dispensary; of group *b* from a sanatorium. The examination was made by palpation of the arm and the presence of a process confirmed by means of the x-ray. The variation in the form of a process was found in four individuals out of 720 adult whites—an incidence of 0.556 per cent of persons having the variation. This is considerably lower than the figure obtained by Terry for dispensary patients (1.16 per cent) and by Cadzow for the insane (1.2 per cent). The examination of a larger group should be made before reaching a conclusion.

21. *The morphology of the dermal glands in nematognathous fishes.* H. D. REED, Zoological Laboratory, Cornell University.

Dermal glands in these fishes are found in every estate of complication from scattered cells to such a complex as occurs in the axilla of most species and in association with the spines of a few. Observations upon the representatives of six families, twenty-six genera, and fifty species lead to the following conclusions: 1) The glands appear to be identical within the group and with the dermal glands of other orders

of fishes so far as investigated. 2) The cells are destroyed in liberating the secretions, the scattered cells moving to the surface often pushing off those of the surface layer. 3) The gland complex involves a thickening of the whole depth of the epidermis. They are not invaginations of a particular layer. Scattered clavate cells become active gland cells, ordinary epidermal cells become the supporting tissue, the corium becoming the gland sheath. 4) The gland duct and lumen are both false, resulting from the destruction of gland cells. 5) Definitive glands found in different species may be arranged in such a progressive series as to form an exact parallel to the developmental stages of a gland complex. 6) The genesis of the rare spine-glands are incidental to spine development. 7) Glandular activity induced artificially and by the growth of fungus upon the surface of the skin and the effects of the secretions upon fungus growing in cultures indicate that the secretions may serve the fish in both a mechanical and chemical way.

22. Body proportions of whites and negroes during fetal development. ADOLPH H. SCHULTZ, Carnegie Laboratory of Embryology.

Most racial differences exist as early in fetal life as the human form makes its appearance. The variability in fetuses is fully as high as in adults. Asymmetries do not begin with childhood, as has been assumed, but can be recognized with certainty from the fourth month of intra-uterine life. The umbilicus shifts to a relatively higher and the nipples to a relatively lower position on the trunk in the course of growth. Compared to the trunk height the limbs grow very rapidly until the end of the fifth month, whereafter their growth slows down, so that at birth the extremities are again relatively shorter. The forearm grows faster than the upper arm and the leg faster than the thigh; in negroes both forearm and leg are relatively longer than in whites. The thumb becomes relatively shorter with advance in development, but is at all stages slightly longer in whites than in negroes. Hand as well as foot are relatively broader in early than in late fetal life and are broader in whites than in negroes. In man the great toe becomes relatively longer and the other toes relatively shorter during development, while in these points the ontogeny of other primates behaves in just the opposite way. The cephalic index decreases with advance in development. The face grows faster than the brain part of the head and is larger in negroes than in whites.

23. The eye-muscle nerves of Squalus acanthias. H. D. SENIOR, New York University.

At the 1912 meeting of this Association, I reported under the above heading that I had been unable to produce retraction of the lens by stimulating the peripheral end of the cut oculometer nerve in *Squalus*, although I had had no difficulty in doing so in the haddock. I stated also that owing to my inability to find in the former animal any muscle fibers in the situation occupied by the m. retractor lentis of the latter, I was forced to the conclusion that the function of the ciliary ganglion of *Squalus* is vasomotor, but not accommodative. Since these observations had no direct bearing upon the problem in which I was interested at that time, no account of them was published and I have nothing to add now to what I said then. I have been, quoted, however, as stating that "no ciliary ganglion occurs" in *Squalus*, and while I am deeply gratified to find my name coupled with that of Stannius in even so

small a matter, I am glad to avail myself of this opportunity of guarding against further misquotation.

24. *Indirect wallerian degeneration.* SUTHERLAND SIMPSON, Cornell University Medical College, Ithaca, New York.

The indirect wallerian degeneration of Van Gehuchten, easily demonstrated in the rabbit, has not been found by the author in the cat, dog, or monkey. Recently an opportunity was offered to test this method on two young male goats. The seventh nerve on the right side was torn across, close to the cranium in each case. One animal was allowed to live for thirty days after the operation; then killed, the medulla oblongata and pons prepared by the Marchi method and sectioned serially. No evidence of degeneration was found in the central portion of the seventh nerve root on either side. The other animal was killed at the end of forty-five days and the brain stained by the Marchi method as before. In this case indirect wallerian degeneration was evident on the side of the lesion (right), but only about one-fourth of the fibers of the seventh nerve root were involved, instead of all fibers as in the rabbit. The probable explanation of the fact that this phenomenon is not demonstrable in all animals is that the cells of origin of the cranial efferent nerve roots are not equally vulnerable and react to nerve fiber injury with different degrees of intensity. In the rabbit the cells have ceased to act as nutritive centers at the end of about fifteen days; in the goat the time required is longer, and in the cat, dog, and monkey it may be longer still; or cell recovery may take place when no fiber degeneration will follow.

25. *On rotation of lateral-line sense organs in Amblystoma punctatum.* L. S. STONE, Yale University, School of Medicine.

When ectoderm-containing rudiments of the dorsal and midbody lateral-line sense organs is rotated 180° after stage 23 by transplanting it from the opposite side and reversing the anterior and posterior poles, the midbody line fails to grow in the normal posterior direction. When similar transplants are reversed somewhere near stage 33 the dorsal body line does not grow posteriorly. When the transplant is taken in like manner as late as stage 33 and rotated 180° in respect to its dorsal and ventral poles the normal path of growth is apparently not disturbed. If the graft is taken from the same side, thus rotating both axes 180°, direction of growth of the midbody line may be affected as early as stage 22-23, that of the dorsal body line as early as stage 26. In cases where rotation affected normal growth, the rudiments grow no further anteriorly than the occipital region where the lateral-line organs increase in number and are irregularly arranged. When the transplant is taken from the same side as late as stage 33 and rotated 90° either anteroventrally or anterodorsally, the normal posterior direction of growth is recovered before the rudiments make their appearance upon the side of the trunk. These lines occasionally follow an irregular course, but in the normal pathway. The ventral body line was normal in all cases through stage 33. Implanted rudiments were followed readily as transplants were made from embryos stained in Nile-blue sulphate.

26. *Post-pubertal ovogenesis in the guinea-pig.* YUN-CHAN SUN, Department of Anatomy, Washington University School of Medicine. (Introduced by E. Allen.)

Ovaries of young, sexually mature guinea-pigs taken at various periods of the

oestrous cycle were studied for evidence of new formations of ova. Near the time of ovulation there are considerable numbers of small ova in and just under the germinal epithelium. Counts of these in eight ovaries vary from 28 to 176 (average 56). The highest count in nine ovaries at other stages of the cycle was 12 (average 2.6). Small ova are numerous in the tunica albuginea during and following the period of ovulation. Their intermediate position between the germinal epithelium and ova underlying the tunica suggests a transition which is supported by the following points: 1) only an undifferentiated type of cell is found in the germinal epithelium during the dioestrous interval; 2) small ova differentiate in this location near the time of ovulation; 3) during and following this period considerable numbers of ova are found in the tunica albuginea, and, 4) there is a gradual increase in size of ova with depth below the germinal epithelium. This is considered evidence that the covering epithelium of the guinea-pig ovary is truly germinal and contributes new ova even after the attainment of sexual maturity.

27. *The phagocytic activity of hemal nodes.* H. H. WOOLLARD and G. B. WISLOCKI, The Department of Anatomy, The Johns Hopkins University.

We have observed the phagocytic power of hemal nodes towards carbon particles injected into the blood stream of the living sheep. Briefly, our results are as follows: Hemal nodes are capable of removing carbon particles from the circulation. The carbon appears in the nodes in the form of coarse clumps within the cytoplasm of large mononuclear phagocytic cells. These cells lie in part free within the venous lacunae, in part in the lymphoid tissue surrounding the lacunae, and less frequently in the so-called blood spaces or sinuses. In addition to the clumps of carbon in these conspicuous cells, carbon granules of dust-like dimensions are observed in some hemal nodes in the cytoplasm of the irregular-shaped reticulum cells which form the mesh-work of the blood spaces. The hemal nodes of the sheep, although capable of removing carbon particles from the blood stream, possess this ability to a very slight degree in comparison with the spleen and liver. Only traces of carbon escape directly from the blood stream into ordinary lymph nodes, but by permitting carbon granules to reach the ordinary lymph nodes by way of their afferent lymphatics, we can demonstrate a far greater capacity to store particulate matter by lymph nodes than by hemal nodes. Lastly, we have shown in the cat and rabbit that accessory splenic nodules, whether large or minute, possess the power to phagocytize carbon granules in the same degree as the spleen itself.

PAPERS TO BE PRESENTED

1. *The ophidian musculature and its origin.* L. A. ADAMS, University of Illinois.

The streptostylic skull of the Ophidia with its muscles gives a very interesting problem in the adaptation of the original reptilian musculature to a highly specialized condition. Since the jaw movements in the reptiles are generally quite simple, there has been little subdivision of the original muscle masses, while in the ophidians with their very highly specialized skull and jaw movements these original muscle masses have divided into slips and individual muscles. The capiti mandibularis becomes split into five or more slips, to function in the complex jaw movements. Some of the ophidian muscles are difficult to homologize with those of the other

Reptilia, since there has been so much shifting of parts. The pterygoid bone develops a complex and highly specialized musculature, due to its importance in the movements of the maxillae and palatal region. The movable quadrate and squamosal develop a special musculature. The sphenoid develops three sets of muscles on the ventral face that are of special importance in the movements of the palatal region. The vomers develop a pair of muscles that probably come as slips from the pterygo-sphenoidalis anterior. The musculature is further complicated by the specialization that occurs in connection with the poison-injecting apparatus of the venomous species.

2. *On the red blood cells of the new-born albino rat.* W. H. F. ADDISON and M. N. RICHTER, University of Pennsylvania and The Wistar Institute of Anatomy.

Both immature and mature forms of the red and white blood cells are found in the circulating blood of the new-born albino rat. The evidence of immaturity in the hemoglobin-containing cells are seen in both their nuclear and cytoplasmic constituents. The variations of nuclear origin, associated with immaturity, are the presence of erythroblasts, normoblasts with and without basophilic stippling, karyorrhexis in normoblasts, nuclear remains (Howell-Jolly bodies) in normoblasts, and extrusion of normoblast nuclei. The cytoplasmic evidences of immaturity are basophilic stippling, polychromatophilia, as well as anisocytosis. These appearances are all to be seen in the cells after fixation. In the living cells the presence of the reticulum or skein is shown in many cells by vital dyes. The total number of red blood cells in the peripheral blood of the new-born albino rat averages 3,000,000 per cu.mm., as found in twenty-three healthy, standard animals. At birth the nucleated red cells are always more numerous than the white cells. In the days following birth the number of normoblasts rapidly decreases, while the number of leucocytes usually increases. Already at two days of age the leucocytes are often the more numerous. Sometimes, however, it is several days later before the normoblasts number less than the leucocytes. The animals giving this picture are usually weak. Thus in one instance a four-day-old marasmic animal, weighing 5 grams (about the weight of a healthy new-born), had the same number of normoblasts and leucocytes as a healthy new-born animal might have had.

3. *Ovogenesis during sexual maturity.* EDGAR ALLEN, Department of Anatomy, Washington University School of Medicine. (Lantern.)

New formations of ova from the germinal epithelium of the mouse ovary occur during sexual maturity. These proliferations are periodical, occurring when the ovaries are hyperemic at oestrus. During the dioestrous interval the process ceases. A description is given of the earliest stages of ovogenesis, viz., mitoses in the germinal epithelium, ova just under it, and ova two cells below the surface. The incidence of these stages is recorded and evidence presented for an actual transition between them. From four to six days (one oestrous period) are required for this transition; 400 to 500 ova differentiate in one ovary at each oestrus. The survival approximates only 1 per cent. Semispaying does not increase the number of ova differentiating in the remaining ovary, but does double the number surviving. The low percentage of survival affords an opportunity for the operation of selection in the genesis of the definitive ova and necessitates the recognition of more extensive degeneration of young ova than required by the accepted theory of ovogenesis. Since investigators

agree that ovogenesis recurs seasonally in fishes, amphibians, and reptiles, this evidence places the mouse in line with the lower vertebrates in this respect. This evidence is in opposition to the theory that the definitive ova are in the direct line of chromosomal descent from the primordial ova. The continuation of ovogenesis during sexual maturity makes this process more analogous to spermatogenesis.

4. *Formulae for calculating the age and size of human embryos.* LESLIE B. AREY, Northwestern University Medical School.

The age of an embryo or fetus can be calculated by the following rules:

Standing height (in cm.) $\times 0.2 =$ Age (in months)

Sitting height (in cm.) $\times 0.3 =$ Age (in months)

(For embryos less than 10 cm. long add one month to the result.)

The size of an embryo or fetus can be calculated by the following rules:

Age (in months) $\div 0.2 =$ Standing height (in cm.)

Age (in months) $\div 0.3 =$ Sitting height (in cm.)

(For embryos of the first three months subtract 4 cm. from the result.)

It will be noticed that the only digits used are 1, 2, 3, 4 and their sum which is 10. Ordinary experience indicates how they should be applied to avoid incongruous results. Consequently, the formulae may be derived at any time without specially taxing the memory.

5. *The function and regeneration of the seminal vesicles of the guinea-pig.* REO B. ARMITSTEAD, Stanford University Medical School. (Introduced by A. W. Meyer.)

Spermatozoa were not normally found in the seminal vesicles. The contained secretion had a definite activating effect upon spermatozoa removed from the ductus deferens, perhaps largely because of its alkaline reaction. Removal of the vesicles in the adult was not followed by regeneration, and ligation of the bases of the vesicles without serious disturbance of the blood supply was followed by an initial enlargement but final atrophy. Removal was found to reduce the number of complete copulations, and hence seems to depress the libido. Excision seemed to greatly reduce if not altogether abolish the fertilizing power of the males observed. A vaginal plug was not invariably present in guinea-pigs after coitus. The epithelium of the vesicles was observed to pass through definite changes during the secretory cycle.

6. *The histological differentiation of the pars tuberalis in the hypophysis of the cat.* WAYNE J. ATWELL, Department of Anatomy, University of Buffalo.

The cat is one of the most favorable animals for the study of the pars tuberalis. Immediate fixation, obtained by injection, and followed by special stains for cytoplasmic structures, serves to differentiate this portion of the hypophysis most clearly from the pars intermedia and the pars anterior propior. Fixatives employed include Zenker-formol, Helley, formol-bichromate, bichromate-osmic-acetic, Bouin, Carnoy and formalin. In addition the Golgi, Cajal, and pyridine silver methods were used. The most valuable stains were found to be safranin-acid violet and phosphomolybdic anilin blue-orange G.

The pars tuberalis of the cat is very vascular. It is not so compact as the other portions of the hypophysis, but resembles the terminal ramifications of an em-

bryonic gland. It does not contain any of the several types of chromophilic cells which are found in the anterior lobe proper. It contains neither the secretory cells of the pars intermedia nor its peculiar supporting cells which the Golgi, Cajal, and pyridine silver methods bring out so beautifully. The pars tuberalis is characterized by numerous small follicles containing a colloid material. The stages in the formation of this colloid from the cells of the part may readily be followed. No definite secretory granules other than colloid droplets have been demonstrated by methods so far employed.

7. *Observations on the development of the cranial ganglia in man.* G. W. BARTELMIZ, Carnegie Laboratory of Embryology and University of Chicago.

In embryos of eight somites when the neural crest begins to proliferate, ectodermal thickenings outline the future visceral region of the head. The crest then increases in bulk and spreads, whereupon the first two visceral arches appear as eminences on the surface. In a 13-somite embryo the crest extends from the midbrain and the first hindbrain segment to a great maxillomandibular ectodermal thickening; a more sharply defined pillar of crest cells extends from the 'fourth' rhombomere to an hyoidean epibranchial placode, and IX and X placodes are also differentiated. In a 16-somite embryo four visceral arches are mapped out and cells are proliferating from the VII and IX placodes. The trigeminal ectodermal plate seems to be disappearing as a result of a similar proliferation. It is conceivable that most of the trigeminal ganglion cells come from this short-lived placode, as is indicated for Amphibia by the experiments of Stone. If his conclusion, that all general cutaneous components of the cranial ganglia come from placodes, proves to be general, it will be necessary to assume that the large contribution of crest to the gasserian ganglion develops mostly into capsular and sheath cells.

8. *The blood supply of muscles.* O. V. BATSON, University of Cincinnati, College of Medicine.

The blood vessels to certain muscles enter through a definite connective tissue band. This band is similar to and continuous with the mesotendon. For this reason the term mesomyon or mesomuscle is tentatively used. This structure is most readily demonstrated in fusiform muscles. The blood-vessel pattern in the band suggests the embryonic distribution. The continuity of the mesotendon and the mesomyon throws light on the functional origin of the synovial sheath.

9a. *Curves of growth.* R. BENNETT BEAN, Laboratory of Anatomy, University of Virginia.

The usual curves of growth give the actual size at specified ages, but true growth is best represented by the percentage increment for specified periods based upon the size at the beginning of the period of growth. Curves of growth were constructed by both methods, for comparison, with illuminating results. The curves of actual size show that growth is absolutely slow during the periods immediately following conception and birth, but rapid during the periods immediately preceding birth and maturity. The curves of percentage increments show the reverse. The relative growth is rapid during the periods immediately following conception and birth, and relatively slow during the periods immediately preceding birth and maturity.

Furthermore, the curves of actual size were almost entirely smooth or regular, but the curves of percentage increments showed a distinct alternation of slow and rapid growth for practically all weights and measurements of the organs and parts of the body. Likewise, when masses of weights and measurements by many authors and including both sexes and different races were grouped together, the curves became smooth, but when the weights or measurements were selected in small groups with each group weighed or measured by one person, and when race, sex, and type could be separated, the alternation in growth became more distinct. Except for the records of Godin, all available material, of any large number of persons, represents cross-sections of groups and not actual growth of individuals; therefore, the results are open to criticism, although they probably represent an approximation to the truth.

9b. *Alternation in growth, preliminary report.* R. BENNETT BEAN, Anatomical Laboratory, University of Virginia.

By using the curves of percentage increments it is found that accelerations and retardations occur in the growth of each organ or part of the body. The acceleration periods are placed tentatively at the following ages:

Stature: prenatal, 1 and 2 mo., 6 and 7 mo., and 9 and 10 mo.; postnatal, 1, 4-5, 7-8, and 13-16 years. *Sitting height:* prenatal, 1 and 6-7 mo.; postnatal, 1, 5-6, and 13-16 years. *Extremities:* prenatal, 2, 9 and 10 mo.; postnatal, 1, 4, 8, and 12 years. *Bone:* prenatal, 6-7, and 9-10 mo.; postnatal, 1, 4-8, 11-16 years. *Head:* prenatal, 2, 6 and 9 mo.; postnatal, 1-4, 7-8, and 11-12 years. *Brain:* prenatal, 1, 5-6 and 8-9 mo.; postnatal, 1-2 and 11-14 years. *Prenata*, the cord and brain stem at 1 and 5, cerebrum at 6, and cerebellum at 9 mon. *Heart:* prenatal, 1 mo.; postnatal, 3, 5-6, 13-14, and 17-20 years. *Liver:* prenatal, 1-3 mo.; postnatal, 1-3, 5-6, 9-10, and 12 years. *Spleen:* prenatal, 7-9 mo.; postnatal, 5-6, 9-10 years. *Kidneys:* prenatal, 3-5, 6-7 mo.; postnatal, 4, and 13-14 years. *Lungs:* prenatal, 4 mo.; postnatal, 1 year, 14-16 years. *Stomach and intestine:* prenatal, 7 and 8 mo.; postnatal, 9 and 10 years. *Muscles:* prenatal, 9 and 10 mo., postnatal, 16-18 years. *Shoulders:* prenatal —; postnatal, 1-4 and 15-16 years. *Pelvis:* postnatal, 7-8 and 15-16 years. *Teeth:* postnatal, 2, 7, 11 and 18 years.

9c. *The pulse of growth, tentative.* R. BENNETT BEAN, Laboratory of Anatomy, University of Virginia.

If a curve of growth is constructed for both the prenatal and postnatal periods and on each of these curves small impulse curves are placed, the latter will represent the pulse of growth. The curves may be arranged for each month of prenatal and each year of postnatal growth.

Prenatal—1 month: Trunk, heart, spinal cord. 2, Head, brain, extremities. 3, Liver, thymus. 4, Kidneys, lungs. 5, Spinal cord and brain stem. 6, Cerebrum, torso. 7, Torso, kidneys, stomach. 8, Intestine, spleen. 9, Cerebellum, muscles, extremities. 10, Muscles, extremities.

Postnatal—1 year: Lungs, torso, extremities. 2, Teeth. 3, Heart. 4, Kidneys. 1-4, Head, brain, shoulders. 5-6, Stature, torso, heart, liver, spleen. 7-8, Stature, head, pelvis, teeth. 9-10, Stomach, intestine, liver, spleen. 11-12, Liver, head, teeth. 13-14, Stature, torso, heart. 15-16, Torso, pelvis, shoulders. 13-16, Brain, lung, muscles. 17-18, Muscles. 19-20, Teeth. 17-20, Heart.

10. *The irruption of injection masses into the hepatic cell.* R. R. BENSLEY, Hull Laboratory of Anatomy, University of Chicago.

Channels in the hepatic cell are found filled with injection mass as described by Schaefer, and by Hering and Simpson. The earliest phase is the production of round and oval droplets located in the protoplasm alongside of the sinusoid with which the droplet communicates by a narrow opening. The final result is staining of the entire cell. An intermediate result is the production of a net-like injection of canals communicating at one or more points with the sinusoid. This result is not dependent on reaction of the injection mass, and is in no sense due to staining of the intracellular substances by diffused dye. The point of richest injection of the liver cell is, however, always the location of the most complete filling of the sinusoid and therefore the locus of the highest injection pressures. While Hering and Simpson produced this result by initial pressures of low degree, they left out of consideration the viscosity of the medium and the obstructions produced locally by retained blood, and by stasis of the injection mass from other causes. The most complete injections of liver cell may be secured by insuring the maintenance of a high pressure in the sinusoids, as, for example, by obstructing the outflow from the vena cava. Furthermore, frequently one finds in a single hepatic cell eight or ten points where injection mass has entered the cell from the sinusoid. There is, therefore, little doubt that these results are due to mechanical irruption of the liver cell.

11. *The problem of the pancreatic bladder in the cat, with new observations on the structure, functional periodicity, and anatomical variations of the gall-bladder noted in twenty-five hundred specimens.* EDWARD A. BOYDEN, Harvard Medical School. (Lantern.)

Accessory bladders occur with extraordinary frequency in the biliary tract of the cat—one to every eight specimens. They fall into three groups, *supernumerary* gall-bladders (extra lobes of the cystic diverticulum), *ductular* bladders, originating directly from the hepatic ducts, and *pancreatic* bladders developed from diverticula of the pancreas or duodenum.

The first group arises from paired or cleft primordia, from epithelial buds or aborted branches of the cystic ducts, and probably from perforation in the irregularly shaped solid stage of the gall-bladder. Supernumerary and ductular bladders have the same histological pattern as the gall-bladder.

The third group is assigned a pancreatic origin on the following grounds: 1) the discovery of one case unquestionably derived from an accessory pancreas (Boyden, '22); 2) the reported absence, in the cat, of partially split ductus choledochi which might be said to bridge the gap between split gall-bladders and pancreatic bladders; 3) the occurrence of forty pancreatic lobes paralleling the bile-duct and duplicating all essential proximal relations exhibited by pancreatic bladders; 4) the frequent presence of inflammatory lesions in pancreatic bladders, including Professor Beckwith's anomaly, from which it is deduced that her case represents an acquired fistulous union, and not an imperfectly split cystic diverticulum; 5) the establishment of an histological distinction between pancreatic and gall-bladders, the mucosa in the former being richly supplied with branched alveolar glands, suggestively like pancreatic acini, which penetrate the tunica muscularis; these are easily distinguished from the broader basal pockets of the gall-bladder which disappear under inflation.

- 12a. *An experimentally produced aberrant olfactory nerve in Amblystoma.* H. SAXTON BURR, Department of Anatomy, Yale University.

Following the transplantation of an additional nasal placode adjacent to the normal olfactory primordium in *Amblystoma*, in twenty-nine instances out of forty-eight operations performed an aberrant nerve has been observed growing medially from the inner aspect of the transplant toward the brain case. In seventeen cases this nerve pierced the wall of the brain case either through cartilage or membrane and coursing caudally along the cerebral hemisphere ended finally in a ganglionic enlargement, through which it was connected to the lateral aspect of the midbrain. In additional cases the nerve, though following the above course, did not establish connection with the midbrain. The remaining embryos showed aberrant olfactory nerves wandering cephalad or caudal along the profundus branch of the trigeminal nerve or snarling in a knot on the medial aspect of the transplant. It is suggested that the establishment of this connection between an ectodermal placode and a region of the brain with which such connection never normally obtains is an indication of a possible relation between the nose and midbrain which usually occurs through secondary or tertiary tracts within the central nervous system. Attention is called to the fact that the distance traveled by the growing point of this aberrant nerve is relatively great, comparable to the distance traveled by spinal nerves to the transplanted limb end as described by Detwiler. The stimulus for this connection must, therefore, be great, arguing a strong normal connection of the nasal apparatus with the midbrain.

- 12b. *Experimental hyperplasia of the cerebral hemispheres in Amblystoma.* H. SAXTON BURR, Department of Anatomy, Yale University.

A study of the effect of the transplantation of an additional olfactory placode adjacent to the normal nasal primordium, on the development of the cerebral hemisphere, was carried out on *Amblystoma* embryos. The operations resulted in all degrees of relationship obtaining between the normal placode and the transplant, from complete fusion to entire independence. Connection with the cerebral hemisphere was established in all instances through an olfactory nerve, though the size varied from a slender bundle of nerve fibers to a nerve comparable in size to the normal olfactory nerve with which it eventually joined. Further experiments will be undertaken to determine whether or not the degree of connection established is in any way a function of the position and orientation of the transplant. The effect of the transplantation on the growth of the cerebral hemispheres varies directly as the size of the connection established. In those instances in which the bundle of nerve fibers from the transplant is large, a definite increase in the size of the adjacent hemisphere is observable. This is apparently due to an augmented number of cells in the olfactory bulb and accessory olfactory nucleus. The operations resulting in slender connections produced no observable gross changes in the hemisphere, though further investigation may show a slight increase in cell counts in the regions above mentioned.

13. *What holds the blastomeres together in the segmenting sanddollar egg?* ROBERT CHAMBERS, Cornell University Medical College.

It has long been known in the sea-urchin egg that, a few minutes after fertilization, a hyaline layer appears on the periphery of the egg which later bridges the cleavage

furrows. It has been assumed that this hyaline layer serves as an intercellular cement. The microdissection needle has demonstrated that this layer is in reality a fluid space covered by a delicate, elastic pellicle. The egg segments within this pellicle. Just prior to the first cleavage the egg is elongated and the two blastomeres, when first formed, are almost spherical. This stretches the pellicle surrounding them. When the asters fade out the two blastomeres become more fluid and the elasticity of the surrounding pellicle asserts itself by pulling the blastomeres together so that the entire egg assumes again its original spherical shape. As cleavage proceeds, this investing pellicle holds the blastomeres together. The blastomeres can be made to slip over one another within the pellicle and, except under abnormal conditions, there are no demonstrable intercellular bridges of protoplasm. When the pellicle is torn the blastomeres fall apart. The pellicle was found to persist to the gastrula stage beyond which the investigation was not carried. A suggestion is offered, based on the presence of this pellicle and on observations made by Conklin, that the blastocoel contains an albuminous material which, by imbibing water and swelling, pushes the forming blastomeres outward against the investing pellicle until the blastomeres attain the epithelial arrangement characteristic of the blastula.

14. *Conditions affecting the histology of the parathyroid gland of the albino rat.* SIMON B. CHANDLER, University of Arkansas Medical School. (Introduced by Margaret M. Hoskins.)

The parathyroids from animals coming from different colonies show pronounced differences. A margin of dark cells (chromophiles) is common in those from the Wistar and Minnesota colonies, very rare in those from our own stock, and always absent from those obtained at Fayetteville. Experiments have been made to try to determine what conditions govern the appearance of these cells and of the eosinophiles found in the Minnesota material.

The glands studied were those from meat-fed animals, lactating females, and animals in which the nervous condition was noticeably different from that of our stock colony. Meat feeding lasted six weeks, starting when the animals were thirty days old. The parathyroids of these rats were indistinguishable from those of controls on a meat-free diet. Neither group showed chromophiles or eosinophiles. The parathyroids of lactating females also showed no peculiarities to differentiate them from those of males or non-lactating females of the same age and colony.

Two groups of rats were used in testing the effect of nervous condition. The members of one were handled till they became very tame. The others were isolated and became much less tame than our ordinary stock. The parathyroids of the tame rats are not peculiar, but among the few wild specimens examined we find almost the only chromophile cells that our own colony has shown.

The evidence presented shows that meat feeding and lactation do not cause the development of chromophiles or eosinophiles, but is insufficient to show the effect of nervous condition.

15. *Experimental elimination of the larval stage in an ascidian.* C. M. CHILD, the University of Chicago.

In the ascidian, *Corella willmeriana*, the eggs develop in the atrial cavity to the tadpole stage, the tadpoles emerge and begin metamorphosis after a few hours. If

early cleavage stages are removed to normal sea-water, the tadpole stage is more or less completely eliminated by a greater or less degree of inhibition of tail development and metamorphosis often occurs without hatching. In presence of sufficient oxygen, the chief factor determining the course of development under these conditions is H-ion concentration. The pH of normal sea-water is about 8, but in the atrium with respiratory current flowing the pH is about 7.4, though with siphons closed it may fall to 7 or lower, in consequence of CO₂ production. Early cleavage stages removed from atrium of well-aerated sea-water of pH 7.3 to 7.6 give 50 to 100 per cent normal larvae as against 100 per cent more or less inhibited forms in normal sea-water. In more advanced stages the effect of such change is less: if made at the beginning of tail development, 50 per cent or more of normal tadpoles usually result. The eggs are so delicately adjusted to the usual pH of the atrium that changes in either direction from about 7.4 inhibit larval development to some extent. Various other agents produce similar inhibitions, and all observed are non-specific and due to differential susceptibility, the growing tail, because of its high rate of metabolism, being more susceptible than other parts to toxic action.

16. Changes in the nuclei of leucocytes in aseptic inflammation in the tadpole's tail.

ELEANOR LINTON CLARK and E. R. CLARK, University of Georgia.

In the study reported in 1920, on aseptic inflammation produced by the injection of minute globules of croton oil into the transparent tail fins of tadpoles, the leucocytes were watched continuously in the living animal. The same cells were seen to migrate from the blood vessels toward the site of the inflammation, where they became stationary, sent out processes, and changed their appearance so as to resemble small fibroblasts, and subsequently, after the extrusion of the croton oil, they withdrew their processes and rounded up, and, still later, resumed their amoeboid activity and wandered away. The studies have been continued and tadpoles fixed and stained at various stages in the process. It was found that these cells, which are typical polymorphonuclear leucocytes during the early stages of the inflammation, exhibit different staining reactions in their later stages, and that, in the spherical stage, their nuclei round up into the form characteristic of mononuclear leucocytes.

17. The ova and ovaries of guinea-pigs. ESTHER B. CLARK, Stanford Medical School. (Introduced by A. W. Meyer.)

Numerous small cysts, some with microscopic diverticula, were commonly present in the ovaries. The structure of some of these graded into what might be termed cyst-adenomata. Parthenogenetic development of intrafollicular ova up to the formation of germ layers, of a possible neural tube and of placental tissue as described by L. Loeb, were never seen, thus confirming Stockard and Papanicolaou. Eight nuclear spindles were observed, several of which were exquisite in form, but they were not only contained in degenerated follicles, but the ova themselves bore evidences of degeneration as shown by the presence of a crenated cell border and rather indistinct chromosomes. Experimentally induced degeneration of the ovaries revealed follicular degeneration as initiated in the thecal layers with later extension to the granulosa. This is exactly the reverse of the sequence in the process of ordinary follicular atresia. No evidence of phagocytosis of the ovum or of the granulosa was encountered.

18. *Observations and experiments upon the spermatozoa of the guinea-pig.* BERNARD A. CODY, Stanford University Medical School. (Introduced by A. W. Meyer.)

The mechanically detached heads of spermatozoa were never found motile. In becoming detached the head usually clung to the body some distance from the break, apparently held there by a ribbon-like portion of the membrane. The detached tail never was found motile unless the point of division was exactly at the neck. Fragments of the tail always were non-motile. Vigorous spermatozoa always progressed in a straight line against the current with the cupped side of the head up, unless the current was too rapid. Motion in a circle was found due to one-sided movements of the tail.

Some evidence was obtained suggesting that spermatozoa were surrounded by a protecting film of the circumambient fluid of the ductus deferentes, which when removed by transfer of the spermatozoa to another medium rapidly hastened death. The spermatozoa were found very sensitive to increases, but not to comparable decreases in the osmotic pressure of the surrounding medium.

Two-tailed spermatozoa were the commonest of the abnormal forms found, but no conclusive evidence was obtained for the existence of two-headed or of dwarf forms. Larger individuals were found only very rarely. Only slight reduction in fertilizing power was found in a series of six males in which the seminal vesicles had been excised by a fellow-worker.

19. *The growth of neuroblasts in relation to physiological gradients and differential rates of metabolism.* G. E. COGHILL, Department of Anatomy, University of Kansas.

The orientation of the earliest neuroblasts in *Amblystoma* conforms to the axial physiological gradients as demonstrated by Child and others, in that the pole of lower rate in the gradient of the neurone grows towards the region of lower rate in the axial gradients of the embryo: the sensory being dominated by the nervous ectoderm; the motor, by the mesoderm. The primary sensory and motor nerves respond similarly to secondary gradients of the ectoderm and mesoderm. The organization of the neurones into functional systems, also, observes the same principle, the pole of the lower rate in the gradient of one neuroblast growing to the pole of the higher rate of another. In later development up to the perfection of locomotion there are periods of acceleration and retardation in the rate of differentiation of neuroblasts in localized regions of the central nervous system, and in several instances there is obvious correlation of acceleration with the growth of axones into the region. Upon the basis of data in hand, it is reasonable to inquire whether relative rates of differentiation may not be the expression of metabolic gradients as conceived by Child, and whether periodic acceleration in rates as secondary gradients, dominating more or less localized regions, may not be the directive agent in the growth of conduction paths. Should this be true, the development and function of the nervous system could be viewed as operating under the same law. The early development of *Amblystoma* is being studied from this point of view.

20. *Factors which govern the direction of epidermal ridges and their arrangement in patterns.* HAROLD CUMMINS, The Tulane University of Louisiana. (Lantern.)

Observations have been made upon epidermal ridge configurations in the following developmental defects: membranous and cutaneous syndactylism, high-grade syn-

dactylism, ectrodactylism, and hyperdactylism, each defect being represented by a number of cases. In addition, an acephalic monster presenting diverse defects of the extremities and one case of intra-uterine dactylosis have been studied.

In so far as it has been possible to ascertain contours, there is a consistent correlation between the contours of the ridge-bearing surfaces and the character of the configurations which they bear, even though the contours and patterns are anomalous. Whorls are found upon completely circumscribed elevations, loops upon distinctly asymmetrical and incompletely circumscribed elevations, and patternless areas upon subcylindrical and flattened surfaces. That this correlation exists independently of any germinal provision is obvious, especially in such material as the acephalus and the infant with dactylosis. In hyperdactylism the occasional lack of correspondence in the configurations of two unquestionably equivalent digits argues against a germinal determination of their patterns. Likewise, the characteristic variants of syndactyls are traced to non-germinal sources. The findings are interpreted to mean that germinal epidermal determinants are restricted to the potencies of ridge formation, delegating the directive control of ridges to other factors, which are in the nature of growth forces.

21. *The status of unilateral variations in man.* C. H. DANFORTH, Department of Anatomy, Stanford University School of Medicine.

The occurrence of unilateral variation in structures normally bilaterally symmetrical raises the question as to whether such variation represents anything more than chance fluctuation in development. That certain structural variations observed in lower forms are to be explained in this way has been suggested, and Sumner and Huestis have recently presented evidence that variations in the attachment of sacrum and ilia in *Peromyscus* are of this class. Possibly some of the variations met with in human anatomy are of the same nature, but an analysis of a collection of personal observations and a considerable series of data from the literature show that a number of common variations (departures from the modal condition) in man appear on both sides of the same body far more often than would be the case if they were due to chance causes affecting the two sides independently. It is statistically demonstrable that the causal factors for many of these abmodalities are such as tend to affect both sides of the body simultaneously. Such evidence as can be brought to bear on the question indicates that these factors are germinal, and the hereditary nature of some of them has been definitely established. In these the transmission seems to be about the same whether the parental manifestation is unilateral or bilateral. The conclusion is reached that there are a considerable number of germinal modifications which frequently fail to produce any observable effect on one side (and perhaps occasionally on both sides) of the body.

22. *The deep sensibility of the face.* LOYAL E. DAVIS, Fellow of the National Research Council, Northwestern University Medical School. (Introduced by S. W. Ranson.)

The deep sensibility of the face was investigated to determine whether or not pressure-pain sensibility was lost after division of the sensory root of the trigeminal nerve and if this type of sensation is not lost, to ascertain whether or not the fibers which transmit pressure pain travel cephalad in the facial nerve.

An intracranial division of the sensory root of the trigeminal nerve or complete destruction and avulsion of the gasserian ganglion was performed upon twenty-five cats. After recovery from the operation, sensation in the face was tested for by the use of an electric current. In each instance there was no response upon the side of the lesion to superficial stimulation over the trigeminal area. If the electrodes were thrust into the muscles of the face and stimulation effected, the response was immediate. At a second operation the facial nerve upon the same side was severed at its exit from the stylomastoid foramen. After recovery there was no response either to superficial or deep stimulation with the electric current.

Deep pressure pain as elicited by the algometer was present without exception in fifteen clinical cases following trigeminal neurectomy, although superficial cutaneous sensibility was lost. Two cases were observed in which there was a paralysis of both the fifth and seventh cranial nerves upon the same side, with loss of deep as well as superficial sensibility.

It is concluded that afferent fibers which transmit pressure-pain sensation from the face pass cephalad in the facial nerve.

23. *On the effect of captivity or domestication on the brain weight of some mammals.*

HENRY H. DONALDSON, The Wistar Institute of Anatomy and Biology.

In the rat, the guinea-pig, and the rabbit—all rodents—also in the lion and the fox, the brain weight (or cranial capacity) is less in the captives than in the wild. The deficiency in captive lions is 19 per cent (Hollister) and in the domesticated guinea-pigs about 7 per cent. Mere captivity may produce a marked effect on animals caged when young. Detailed studies on the rat show that the olfactory bulbs are specially reduced. A defective diet increases this effect. The growth of the brain and bulbs is stimulated by exercise. In the rat the lower weight is not due to a loss of neurons, but to a reduction in their size (Holt, Sugita) and this reduction appears to involve the entire neuron as the percentage of water is not modified. Captivity has apparently a greater effect in the cases where the departure from wild habits and diet is most marked. Whether this retardation in growth is direct or indirect is undetermined.

24. *The angle of gait.* STANLEY DOUGAN, Stanford University Medical School. (Introduced by A. W. Meyer.)

Although some persons toe in on one side and out on the other, very few persons toe in on both sides. Only two out of 103 high-school and one out of 126 university students toed in on both sides, but thirteen of the former and nine of the latter toed in on one side. Only one person in each group walked wholly without deflection of the feet.

Of the combined series of 229 males eight toed in on the right side and fourteen on the left. The most striking thing observed was the great disparity in the angle of gait on the two sides in the same individual, and also the inconstancy of the angle on the same side during successive steps. Only 6 individuals out of the 229 had exactly the same angle of gait on both sides, if allowance is made for an error of measurement of one-tenth of a degree.

Among university students, including graduates, the greatest angle on the right was 20.3° and on the left 16.5°. Among high-school students these angles were 21°

on the right and 16° on the left. The largest negative angle in university men was 5.8° on the right and 6° on the left, making a total range of 26.1° on the right and 22.5° on the left. Among high-school boys this range of action was 24.2° on both right and left. The largest negative angle in these boys was 3.2° on the right and 8.2° on the left. The average angle in the 126 university students was 7.55° on the right and 6.59° on the left, and in the 103 high-school students, 7.25° on the right and 4.87° on the left.

25. *The occurrence and significance of the 'myeloblast' under normal and pathologic conditions.* HAL DOWNEY, Department of Animal Biology, University of Minnesota.

Extensive investigation of normal and pathologic human and mammalian blood and blood-forming organs, particularly by the dry-smear method, has shown that the 'myeloblast' (hemocytoblast, lymphoidocyte), identical with that of leukemic blood, occurs only in the marrow of the normal adult. These cells are not very numerous in the adult marrow, because regeneration is largely homoplastic. They are far more numerous in the marrow of the young and new-born. Similar cells are found in lymph nodes of new-born rabbits, but never in the nodes of normal adults. In the normal adult the regeneration of lymphocytes is, therefore, independent of that of the myeloid cells.

In pathologic cases the myeloblast may serve as a stem-cell for all types of blood cells. This is demonstrated in a case of mixed leukemia in which the myeloblast is related to both the lymphocytes and myelocytes, in a case of lymphatic leukemia in which myeloblasts are numerous in the blood and in the follicles and germ centers of the lymph nodes, and in another case of acute lymphatic leukemia in which the lymphocytes of the blood smears can be traced back to a cell identical with the myeloblast. Dedifferentiation of the lymphocytes and derivation from the reticulum without differentiation are responsible for the appearance of the myeloblast in the lymphatic leukemias. In experimental myeloid metaplasia lymphocytes can differentiate into granulocytes without going through the myeloblast stage.

26. *Growth of the gray and white matter of the cerebral hemispheres from the third fetal month to adult life.* HALBERT L. DUNN, Department of Anatomy, University of Minnesota. (Lantern.)

The volumetric study of the integral parts of the cerebral hemispheres was undertaken on a series of thirty brains ranging in age from three months to adult life. The brains were cut into vertical sections about 3 mm. in diameter and tracings made of all sections. The areas of the gross structures were measured by means of a planimeter and the volumes computed mathematically. By graphic analysis of these data, growth curves of the gray and white matter in the respective subdivisions of the cerebral hemispheres were obtained.

27. *The significance of palpable lymph nodes in the new-born infant.* LOUIS DUNN and HALBERT L. DUNN, Asbury Hospital, Minneapolis, and Department of Anatomy, University of Minnesota. (Lantern.)

The lymphoid organs are relatively large at birth and certain of them, including the superficial lymph nodes, show distinct neonatal changes. This study was

undertaken to determine the factors causing the enlargement of lymph nodes within the first ten days of postnatal life. Three-fourths of the inguinal nodes, one-fifth of the occipital, one-twentieth of the cervical, and none of the mental, cubital, and axillary glands were enlarged in the first two weeks after birth. Functional activity undoubtedly plays a role in the neonatal enlargement of lymph nodes. Infection was found to be the most frequent cause of the cervical node enlargement, while the absorption of a sterile foreign protein was responsible for the majority of palpable occipital and inguinal glands.

28. *Leucocytes and lactation.* V. E. EMMEL, University of Illinois, College of Medicine.

Contrary to a prevalent statement that no definite leucocytic changes occur in relation to the functional activities of the mammary gland, the present paper will advance evidence showing that a characteristic leucocytic reaction can be clearly demonstrated. Under certain conditions during lactation in the rat, a reduction of as much as one-half to two-thirds of the total leucocytic content of the circulating blood may take place. A further analysis of this apparently hithertofore unknown lactation leucopenia will include a comparative consideration of leucocytic behavior under various physiological conditions, together with a correlated study of cytological activities within the active mammary gland.

29. *Independence of the oestrous or heat hormone from that causing 'oestrous' structural changes in the vaginal mucosa of the rat.* HERBERT M. EVANS and K. SCOTT BISHOP, Anatomical Laboratory, University of California.

In the rat, the normal occurrence of cornified cells in the vaginal smear is exclusively associated with the true oestrous and immediate postestrous interval, i.e., only with the occurrence of the oestrous wave. Our present knowledge makes it seem probable that the whole cycle of oestrous changes in the behavior and structure of the animal is determined by cyclic functional and structural changes in the ovary. It is not yet known precisely what ovarian structures are responsible for the cyclic changes in some organs or with the sudden change in the behavior of the animal. Copulation is never accepted after leucocytes appear in the vaginal smear; ovulation has always occurred when the vaginal changes have reached this stage; it is hence clear that oestrous excitement is not due to function of the corpora lutea, but to ovarian structures which reach their functional height before the corpus luteum comes into being. On the other hand, the rapid growth, cornification, and beginning desquamation of the vaginal mucosa always occurs at the time copulation is accepted. The same ovarian structures might conceivably determine both phenomena. This does not appear to be the case. Animals on diets low in vitamin A invariably show continued cornification and desquamation of the vaginal cornified cells, but they do not exhibit continuous oestrus. If the severity of the ovarian lesion is not too great, true oestrus and ovulation may occur irregularly or regularly, independently of the continuous vaginal changes. In other instances oestrus as determined by copulation never occurs. Thus we have witnessed fifty negative tests of this response in an animal exhibiting continuous desquamation of cornified cells (W8618: Diet—whole wheat 66, skim milk powder 33, NaCl 1). It is evident that the ovarian conditions causing cornified changes in the vaginal mucosa of the rat are not identical with those causing oestrous behavior, even though these phenomena are normally associated.

30. *The cause of reproductive upset in dietary deficiencies due to lack of vitamine A.* HERBERT M. EVANS and K. SCOTT BISHOP, Anatomical Laboratory, University of California.

It has been established that the fat-soluble vitamine A is needed in larger quantities for reproduction and lactation than for growth and general health (McCollum, Sherman). It is not known what part of the reproductive mechanism is disabled by deficient A vitamine. We have already called attention to the occurrence in all cases of serious A deficiency of atypical ovarian function causing the continuous production and desquamation of cornified cells by the vaginal mucosa (Evans and Bishop, Anat. Rec., vol. 23, p. 17, 1922). It is conceivable that the implantation mechanism or placental function are also impaired or, indeed, more seriously impaired than the ovary. We have conducted several hundred experimental matings of females maintained on diets deficient in the A factor. In spite of the characteristic ovarian dysfunction, normal follicles may for a time continue to mature so that oestrus and copulation may occur. The continuous vaginal desquamation prevents recognition of the cycle so that experimental mating must be conducted daily. In such cases, however, copulation, when ultimately accepted, may not be followed by other embarrassment in the reproductive process, although continued evidence of lack of vitamine A is afforded by the continuation of cornified vaginal desquamation. In such circumstances fertilization may occur and placentae become established, as shown by the physiological leakage of blood on the fourteenth day of gestation—the 'placental sign.' Such cases come to term. Fewer young are born, but they are usually of normal weight, indicating good foetal growth and hence good placental function. Autopsies late in gestation show a normal implantation percentage (proportion of corpora lutea to implantation sites), i.e., the reduction in the number of young born is not due to failure to implantation, but to the fact that fewer eggs are delivered per ovulation. In more severe instances of the disorder, either from the absence of ovulation or from defective germ-cell vigor, implantation does not occur (the sign of placentation being absent). One is unable to encounter instances in this disease in which after the placental sign is given the birth of litters does not follow. Reproductive disorder due to A deficiency is consequently radically different from that due to lack of the unknown factor X, which we have recently shown to occasion sterility by a disorder which nevertheless permits the placentae to be established, but in which the placentae are diseased (Evans and Bishop, Science, Dec. 8, 1922). When reproduction fails in A disease this is caused by impairment of germ-cell vigor by a selective ovarian, in contrast to uterine, disorder.

31. *Pigment in relation to tissue differentiation in Amblystoma.* HERVEY S. FARIS, Department of Anatomy, University of Kansas. (Introduced by G. E. Coghill.)

The yellowish brown pigment that is well known to be widely distributed through the tissues of amphibian embryos is found to be particularly abundant in regions that are differentiating rapidly. With increasing age of the embryo the granules collect into masses or pigment bodies in the somatic muscles. These bodies form in the middle third of the myotome and increase in size and number as the myotome differentiates. In the central nervous system, except in certain cells, it has a general distribution in fine granules which are exceedingly numerous in lateral and ventrolateral regions of the rhombencephalon and in the lateral regions of the spinal cord,

extending caudad according to the age of the embryo. Cytological and micro-chemical studies show that these pigment granules are formed in the cytoplasm around the nuclei and yolk globules. They do not stain with the ordinary histological stains. They react differentially to neutral red and sudan III in their early period of formation, but only faintly or not at all in the later periods. In their later development they stain with Nile-blue sulphate. While this pigment may, through chemical modification, develop into definitive pigment in certain organs, these studies indicate that it is essentially embryonic in nature and that it characterizes tissue differentiation under the peculiar conditions prevailing in amphibian embryos.

32. *I. Determining the part of the alimentary canal which absorbs fat; and, II. Testing the digestion, absorption and assimilation of the pure fatty acids by the aid of sudan III.* SIMON H. GAGE, Ithaca, New York.

I. From the persistence of the sudan III stain in fat, this dye serves to show with certainty the path taken by the fat in passing from the intestinal lumen along the lacteals, through the lymph nodes and finally the truncus intestinalis to the cisterna chyli. As shown in numerous specimens of cats at different ages, the fat absorption is shown to be from the caudal end of the duodenum, the whole of the jejunum and ileum and for about 12 cm. of the colon. No fat absorption has ever been demonstrated by this method from the stomach, or from the duodenum between the opening of the bile duct and the stomach, nor from the caudal part of the large intestine. In all the animals investigated (cat, dog, rabbit, rat, horse, and sheep) the main absorption is from the middle region of the small intestine.

II. Absorption and assimilation of fatty acids.—The three fatty acids used (oleic, palmitic and stearic) were saturated with sudan III. Stearic and palmitic acids were melted over a water-bath and the sudan added. Cats and rats were fed one of the stained acids mixed with white of egg, and boiled rice, and flavored with beef extract. The feeding was continued for five to seven days. The height of absorption was only after eight to ten hours instead of three or four hours as when butter was fed. Both with cats and rats the perirenal and other adipose tissue became pink after each of the fatty acids. The pure pink fat was obtained by drying the adipose tissue and extracting with ether.

33. *Afferent impulses in the trigeminal nerve.* MARGARET WILSON GERARD, Northwestern University Medical School. (Introduced by S. W. Ranson.)

In thirty-nine cases of occlusion of the posterior inferior cerebellar artery, collected as reported in the literature, it is found that loss of pain and temperature sensibility in the face is always correlated with a destruction of the spinal trigeminal tract or nucleus. Conversely, a case of syringomyelia associates a loss of tactile sensibility with a destruction of the main sensory nucleus.

Experimentally, it is found (in cats) that reflexes due to painful stimuli (struggling, dilation of the pupil, and rise in blood pressure) are lost upon severance of the spinal fifth tract, while tactile reflexes (sneezing) remain intact as long as the path of the main sensory nucleus is not injured. These results indicate that the spinal nucleus is concerned with pain and temperature stimuli, while the main sensory nucleus is concerned with tactile stimuli.

Since the fibers passing through the fifth nerve bifurcate, one branch destined for the main sensory nucleus and the other for the spinal nucleus, it is suggested that all types of impulses may run through a single fiber, but are sifted at the synapses in the various nuclei, those arising from tactile stimuli being relayed from the main sensory nucleus, and those for temperature and pain being relayed from the spinal nucleus.

34. *Cortical lamination pattern of the opossum.* PERCIVAL ALLEN GRAY, JR., Anatomical Laboratory, University of Chicago. (Introduced by C. Judson Herrick.)

The lateral surface of the hemisphere of the Virginian opossum offers two sulci to the unaided eye: 1) a rhinal fissure lying for the most part in the horizontal plane, and, 2) a small sulcus at about the junction of the anterior and middle thirds of the cortex lying above the rhinal fissure. Although somewhat variable in position, this sulcus lies mostly in the vertical plane arched concavely to the front. A faint impression is occasionally visible on the medial surface in the position of the callosomarginal fissure of other mammals. As far as the survey has gone, it seems that the neocortex may be divisible into an anterior agranular and a posterior granular zone. The agranular zone lies for the most part in front of the small sulcus mentioned above. This zone seems to be separable into an anterior region in which all of the cellular elements appear intermingled and a posterior one in which moderately large pyramidal cells may be found. The parietal and temporal regions appear to be characterized by the retention of the typical six layers of Brodmann. Several subdivisions exist, but their positions are at present undetermined. The occipital pole appears to be capped by a type of cortex in which there is a greatly broadened granular layer. This suggests Brodmann's unistriate type of area striata. A special type of cortex may be found in the region of the claustrum which suggests an insular area.

35. *The cerebrum of the opossum.* PERCIVAL ALLEN GRAY, JR., and EDWARD L. TURNER, Anatomical Laboratory, University of Chicago. (Introduced by C. Judson Herrick.)

An extensive study of the brain of the Virginian opossum is in process in this laboratory. At present the following lines are being actively pushed: 1) a histological survey of the lamination pattern of the cortex; 2) a mapping of the electrically excitable region of the cortex, and, 3) a study of the extent of the pyramidal tract by the degeneration method. A number of experiments have been performed and a considerable amount of material collected. This is being studied as rapidly as opportunity permits. The results of previous investigators concerning the extent of the excitable area in this and other marsupials have been confirmed in general in regard to the front leg and face regions. The most unique feature of the study thus far has been our inability to locate a cortical center for hind-leg and tail movements. Toward the solution of this difficulty we are at present devoting our main attention. Diagrams of the excitable area and the lamination pattern within it, so far as our studies reveal them, will be exhibited.

36. *On the possible movement of the stapes.* IRVING HARDESTY, Department of Anatomy, School of Medicine, Tulane University.

In removing the bony labyrinth of the adult pig by approach from the cranial cavity, quite frequently the stapes in place is removed with the labyrinth without

injury to the stapedovestibular articulation (tympanostapedial syndesmosis). A bristle thrust between the crura of the stapes and sprung to rest upon the bone of the two sides of the fenestra vestibuli (ovalis) was used to exert stress outwardly upon the stapedovestibular articulation. Hog bristles of varying thickness were used. For inward stress a fine thread was wound about the specimen and upon the capitulum of the stapes. Sections parallel with the two crura were made of a number so treated and of others fixed with no stress upon the stapes.

The stapedovestibular articulation consists of a continuation of the periosteum of the basis of the stapes with that of the wall of the fenestra in the form of radiating connective-tissue fibers. In no case had the stress of bristle or thread ruptured these fibers. The basis of every stapes not subjected to stress appeared in neutral position, the fibers of the piston-joint radiating parallel with the base, while those fixed under stress were displaced outward or inward, the fibers of the joint being drawn obliquely. The variations were so slight in the excursion from the neutral position as to suggest, since the variations in stress exerted were necessarily greater, that the maximum motion of the stapes in functioning may be fixed. The outward (tympanumward) excursion of the anterior heel of the basis (foot-plate) averaged greater than that of the posterior heel, while the inward excursion of the posterior heel averaged greater than for the anterior heel. The total excursion, inward plus outward, of the anterior heel averaged $52.1\ \mu$, while the same for the posterior heel was $49.6\ \mu$. The total average excursion, inward plus outward, of the entire foot-plate was about $51\ \mu$.

Considering the computed amount of excursion possible in the tympanic membrane, this amount of motion of the stapes may seem small when it is remembered that the incudomalleolar articulation is a purely synarthrosis in the pig, there being no joint cavity, the articular surfaces of the two bones being abundantly joined throughout by connective-tissue fibers which allow but slight joint motion observable in the fresh. The incudostapedial articulation, also examined, likewise allows very little free motion.

37. *Sketch of the evolution of the cerebellum.* C. JUDSON HERRICK, Anatomical Laboratory, University of Chicago.

Comparative anatomy and embryology agree in indicating that the cerebellum arose within the rostral end of the vestibular nucleus under the influence of afferent fibers of other proprioceptive systems derived from the spinal cord and midbrain. The diverse forms assumed by this organ in fishes are correlated with differences in the relative parts played by the component functional systems in different species—vestibular, lateral line, spinal, bulbar, mesencephalic and diencephalic afferent systems. This implies a sort of functional localization in terms of the cerebellar area of distribution of each of these systems of afferent fibers. There is probably, in higher vertebrates at least, also an ill-defined localization of function correlated with the place of origin within the cerebellum of systems of efferent fibers related with diverse synergic groups of muscles. These two localization patterns are not coextensive. The cerebella of amphibians, reptiles, and birds have differentiated in a different direction from those of fishes. In mammals there is added a progressively increasing participation in cerebellar functions of influences related with the cerebral cortex, with consequent profound change in cerebellar organization.

38. *The volume of the normal male adult hypophysis and its various parts.* RUTH HERRICK and A. T. RASMUSSEN, University of Minnesota.

The gland was either put into a vial moistened with strong commercial formalin or directly into 30 per cent formalin. There is no change in weight under these conditions for several hours or even overnight. The weight was obtained after removal of the dural sheath and cutting off the stalk. After fixation in 30 per cent formalin and embedding in paraffin, serial sections were cut 10 μ . One set consisting of every twentieth section was stained with Mallory's connective-tissue stain and another similar alternate set was stained with hematoxylin and eosin. Formalin fixation and Mallory's connective-tissue stain gives an unusually good differential stain for cell types. Sections were outlined at a magnification of 20 diameters on paper whose weight per sq.cm. is fairly constant. Capsule, parenchyma, trabeculae, and large colloid masses in pars anterior, parenchyma, and colloid in pars intermedia and pars nervosa were traced, cut out, and the paper areas weighed. This was found to take less than half the time necessary to determine the areas by the planimeter method. Knowing the thickness of the sections, magnification and weight per sq.cm. of paper, the volume after shrinkage is obtainable. The shrinkage may be as great as 38 per cent. Pars anterior and pars nervosa show practically the same shrinkage. Upon the assumption that this is true of all parts, the percentage of any part may be determined directly from the paper weights. The data resulting from such an analysis of male hypophyses from nineteen to sixty years of age will be presented.

39. *Measurements on the human auditory ossicles.* IVAN C. HERRON, Stanford University Medical School. (Introduced by A. W. Meyer.)

The dimensions of the auditory ossicles and the magnitude of the angles made by certain of their constituent parts, such as processes and crura, with the rest of the respective bones showed that both are subject to variations comparable to those in the larger bones of the human body. The differences found on the two sides of the body were slight and inconstant. No direct relationship was found to exist between variations in the length of the processes of the incus and the size of the entire bone, or between the angles formed between the processes and the body. The height of the opening of the stapes was found to vary independently of its width and the width of the crura varied more than that of the opening. The head of the stapes frequently was sessile.

The most interesting thing encountered in this study was the incision on the medial surface of the posterior process of the incus. Although unmentioned in texts and handbooks on anatomy, it was found present in some form in thirty-six out of sixty-four inci. The foveolae located on the medial and lateral sides of the incus were almost as common, but seem to have remained equally unnoticed.

A set of ossicles from a full-term fetus were found larger than some sets from adults, thus confirming the statement of Allen.

40a. *The hyaline bodies of the posterior lobe of the hypophysis.* MARION HINES, Department of Anatomy, University of Chicago.

The recessus infundibuli of the opossum extends into the pars nervosa. Branching outward from this lumen connective-tissue septa divide the body of the lobe into

organ which is subject to considerable motor activity, with resulting changes in size and form. The appearance of the cells comprising the myenteric plexus can be changed at will to match almost any type described by simply changing the angle at which the tissue is cut, or the method of its fixation. There is no microscopic evidence for the existence of a true nerve net, although there is a most beautiful connective tissue net-work. Axones of the ganglion cells of the myenteric plexus have been traced to terminations in the adjacent muscle layers. Nerve fibers of extrinsic origin form a fine intercellular plexus in the myenteric plexus, similar to that described for other sympathetic ganglia. This plexus disappears upon degenerative section of the vagus and splanchnic fibers, which pass to the gut along the mesenteric vessels.

44. *Survey of forebrain morphology.* J. B. JOHNSTON, University of Minnesota.

Sensitive ectoderm on the dorsal surface of vertebrate embryos, encircling the blastopore, represents the future nervous system (exclusive of the sympathetic system?). Most of this, but not all, is included in the neural plate bounded by neural folds. From the fused lips of the blastopore (notochordal plate) is formed the floor-plate of the neural tube (Kingsbury). The basal plates of His are confluent around the anterior end of the floor-plate in the region of the oculomotorius nuclei and are bounded rostrally by the tuberculum posterius. The alar plates of His, also confluent across the median line, are bounded rostrally by the terminal ridge in which the optic chiasma develops. The whole of the telencephalon and diencephalon is formed from the alar plates. The optic vesicles arise wholly within the somatic receptive laminae. The general pallium and hippocampal formation are both present in selachians. The general pallium has expanded very rapidly in terrestrial animals, while the olfactory apparatus has been relatively reduced in size and has entered into close correlations with the general pallium. The basal area of selachians gives rise to the tuberculum olfactorium, the anterior perforate space, and the diagonal band. The formation of the temporal pole results from a process of moulding of parts already present in the brain of selachians under the influence of the expansion of the general pallium and internal capsule, together with the expansion of the amygdaloid complex and the correlation of the two.

45. *An experimental study of the spleen of the frog, *Rana pipiens*.* H. E. JORDAN and C. C. SPEIDEL, Medical Department, University of Virginia.

Except for a brief period immediately following hibernation, when the bone-marrow is hemopoietically active, the spleen constitutes practically the sole hemopoietic organ of the adult frog. It combines lympho- granulo-, and erythropoietic functions. Small variable collections of lymphocytes occur also in the intestine and kidney. Here they may differentiate into granulocytes, predominantly eosinophils. Splenectomy, partial or total, results only in regeneration of the spleen. It does not stimulate the quiescent bone-marrow to hemopoietic activity. Fifty days after total splenectomy the frog has developed a new spleen of normal size and histologic structure. Experimental hemorrhage likewise has no effect upon the bone-marrow or kidney. It simply increases the hemopoietic activity of the spleen. After removal of approximately 3 cc. of blood, by three aspirations of the heart, at five-day intervals, the spleen is either completely drained of the lymphocytes or it contains many large spheroidal nodules of lymphocytes in various stages of disintegration.

The histologic conditions of the spleen are interpreted in terms of hemopoietic response on the part of the splenic lymphocytes in an effort to compensate for the experimental withdrawal of blood.

The administration of 2.5 mgs. of saponin via the dorsal lymph sac produces effects in the spleen similar to those produced by experimental hemorrhage. The experimental results support the conclusion drawn from a histologic study of the normal spleen namely, that the chief function of the spleen inheres in its content of lymphocytes which supply the progenitors of the various blood cells.

46. *Experimental shift of the locus of hemopoiesis in the larval frog by the use of thyroid extract, and a consideration of the part played by leukocytes in the mechanism of metamorphosis.* H. E. JORDAN and C. C. SPEIDEL, Laboratory of Histology and Embryology, University of Virginia.

In the larval frog the kidney is the chief organ of blood formation, the spleen being of minor importance. In the adult frog the conditions are reversed, the spleen being practically the sole hemopoietic organ, the kidney playing little part. By feeding tadpoles with thyroid extract, thus inducing metamorphosis, the chief locus of hemopoiesis may be shifted from the kidney to the spleen. Thyroid treatment is followed by a drainage of erythrocytes and leucocytes from the kidney. The spleen is stimulated to greater activity. Eosinophils and phagocytes gather in regions destined to undergo regressive change, such as the intestine and tail. Lymphocytes gather in regions of growth and differentiation, such as the limbs. The successful progress of metamorphosis depends largely upon the ability of the lymphocytes, functional as progenitors, to meet the increased demand for, 1) erythrocytes to keep pace with the increased oxidation; 2) phagocytes and eosinophils to aid in the absorption and phagocytosis of parts no longer needed; 3) leukocytes to stimulate parts that are to undergo growth and differentiation. The stimulating effect of leukocytes upon adjacent tissues, as demonstrated by Carrel's experiments with tissue cultures, is due principally, if not entirely, to their lymphocyte component.

47. *The vascularity of the enamel organ in mammals: a comparative study.* H. M. GINGERY, Department of Anatomy, School of Medicine, University of Colorado.

Several papers have recently taken up the question of the blood supply of the enamel organ in developing teeth, and inasmuch as these papers have not agreed, it would seem that the question is not yet settled. A study is being made of the enamel organs of developing teeth in several different forms, and while the work is not complete the results are thought to be of sufficient interest to be presented here. In the white rat, in the rabbit, in the pig, and in man, blood vessels have been found in the enamel organs of developing molars. The blood vessels have been found in teeth in which the enamel is in process of development, more or less well formed, which makes probable the suggestion of Addison and Appleton that the vascularity is correlated with amelogenesis.

48. *Internal migration of the ovum in the guinea-pig.* PAUL B. KINNEY, Stanford University Medical School. (Introduced by A. W. Meyer.)

One ovary was removed in a series of twenty-five guinea-pigs and the pigs mated upon recovery. No implantation was found upon the operated side, although

every care was taken to use only perfectly normal animals and although recovery from the operation was rapid, wholly uncomplicated, and the uterus on the operated side was found practically undisturbed at necropsy.

49. *Is the enteric nervous mechanism essentially a reflex system?* ALBERT KUNTZ and J. E. THOMAS, Department of Anatomy and Physiology, St. Louis University School of Medicine.

Persistence of the rhythmic contractions in the small intestine, gastric peristalsis, and colonic antiperistalsis in the presence of sufficient nicotin to abolish conduction through synapses has seemed to require a nervous mechanism other than a true reflex system. We have demonstrated all the types of contractions named above in the stomach and intestine of intact animals (dog) and in excised pieces of the small intestine (dog, cat, rabbit) after administration of nicotin sufficient to abolish responses to electrical stimulation of postganglionic sympathetic fibers or relatively large doses of adrenalin. Our tracings show that these contractions are normally subject to nervous control. As the nicotin paralysis progresses, tonus and amplitude become more constant until apparently the inhibitory mechanism ceases to function when the amplitude increases suddenly and markedly. Contractions now continue on a constant tone level with diminishing amplitude until adrenalin and electrical stimulation of postganglionic fibers are no longer effective, after which they continue with uniform amplitude. Obviously, the irregularities in rhythm, tonus, and amplitude constantly occurring in unpoisoned preparations are of nervous origin. During the progress of nicotin paralysis the terminal neurons are probably stimulated by the nicotin. However, we need not assume that these neurons are stimulated directly, under normal conditions, since many of them are components of reflex circuits. Consequently, there are no experimental observations which are incompatible with the theory that the enteric plexuses are essentially a reflex system.

50a. *Smooth muscle nerve-spindles.* O. LARSELL, University of Oregon Medical School.

Nerve terminations which differ from motor endings are found in the smooth muscle of the bronchi in dogs and rabbits. The main nerve fibers which lead to a group of such endings divide into branches which are distributed to various points on the smooth muscle bands. These secondary fibers then give rise to spindle-like endings by breaking up into numerous short terminal branches. In a given termination, some of the final branches wrap about the muscle band to terminate upon it as knobs or plates. Other branches in the same spindle penetrate between the individual smooth muscle cells, to terminate among them also in knobs or leaf-like expansions. The structure as a whole suggests the neuromuscular spindles of striated muscle.

50b. *The cerebellum of the frog.* O. LARSELL, University of Oregon Medical School.

The frog's cerebellum shows considerable advance over that of urodele amphibia. Well-developed Purkinje cells and granule cells are present. Basket cells and other elements of higher cerebella have not been found. A continuation of the tegmentum into the body of the cerebellum appears to foreshadow the dentate and roof nuclei. A well-developed nucleus placed laterally in the cerebellar segment, just dorsal to

the level of the sulcus limitans, has connections which suggest the secondary visceral nucleus of fishes. Fibers from the trochlear nerve pass directly into the cerebellum. These appear to be related to the muscle-sense system. In addition to the mesencephalic V tract, which passes through the cerebellum, there is clearly evident a direct trigeminocerebellar tract. The lateral-line system is absent in the adult, but the vestibular connections are well developed. There is a vestibulocerebellar as well as a direct cerebellar path. Other connections include spinocerebellar, bulbo-cerebellar, tectocerebellar, and cerebellohypothalamic tracts, besides brachium conjunctivum and arcuate fibers (cerebellomesencephalic and bulbar). The disappearance of the lateral-line system is compensated for by the greater development of the vestibular connections, and apparently to some extent by inclusion of muscle-sense fibers. The auricular lobe is present in relatively reduced form as compared with urodeles. It receives vestibular fibers.

51. *The growth of the skeleton and some of the individual bones of the single comb white Leghorn chicken.* HOMER B. LATIMER, University of Nebraska.

The weights of the moist and the oven-dried cartilaginous skeletons of ninety-seven chickens (from hatching to maturity) plotted on gross body weight show a sex-difference. The weights of the humerus, tibia, and tarsometatarsus show a sex-difference. The radius, ulna, and femur show no distinct sex-difference in weight. Empirical formulae have been prepared for all these curves and will be presented at the meeting.

The water loss in the oven dried skeleton varies from about 70 per cent in the youngest to about 38 per cent in the adults. The corresponding water loss in the air dried skeleton is 66 and 32 per cent.

The percentages of the net body weight of the entire skeleton and of the six bones show a rapid rise between hatching and 200 grams gross body weight. The weights of the six individual bones reduced to percentages of the entire oven-dried skeleton remain nearly constant. The values are as follows: femur, 11.43; tibia, 14.79; tarsometatarsus, 8.23; humerus, 7.62; radius, 1.61, and the ulna, 4.82 per cent. The tibia and the tarsometatarsus form a higher percentage at the very first and the bones of the wing are relatively lighter. The relative weights of the femur do not show this early fluctuation. The lengths of these six bones when plotted on body weight form curves which are convex superiorly. The ossification is completed earlier in the females than in the males.

52. *The topography of the viscera in the chicken.* HOMER B. LATIMER and JOHN L. OSBORN, University of Nebraska.

The topography of the viscera of the adult chicken has been studied by means of the orthoscopic drawing apparatus described by Scammon. All the specimens studied thus far show a fairly constant relationship of most of the organs. The lungs extend from the level of the sixteenth cervical to the fifth thoracic vertebra. The apex of the heart is found at the lower border of the fourth thoracic vertebra and slightly to the left side. The liver extends from the level of the second thoracic vertebra, ventral to the lungs, to about the level of the acetabulum. The glandular stomach opens into the gizzard, or ventriculus, on the left side, at the level of the tip of the iliac process. The gizzard is more nearly perpendicular in the males.

In the females it appears to be pushed ventrally and obliquely to the right by the ovary. The intestines vary, with the exception of the duodenal loop which encloses the elongated pancreas. The terminal portion of the intestine is more constant in its relations. Two bile ducts and three pancreatic ducts open into the distal end of the duodenal loop in a fairly constant manner. The three-lobed kidneys are irregular in shape and extend caudad from the level of the last thoracic vertebra. The two testes are parallel and extend obliquely caudad and toward the right side. Their anterior ends are at the level of the fourth thoracic vertebra.

53. *The variation in weight of young chickens and of some organs in adult chickens.*
HOMER B. LATIMER and LOIS T. PEDERSEN, University of Nebraska.

The hatching weights of 966 Single Comb White Leghorn chickens and the weights of 795 of the above chickens at thirty-five days were obtained from the Department of Poultry Husbandry, University of Nebraska. At the time of hatching 513 males averaged 39.22 ± 0.09 grams, and the 453 females averaged 39.07 ± 0.09 grams. The coefficient of variability for these males is 7.44 ± 0.16 , and for the females, 8.31 ± 0.19 . When thirty-five days old, 416 males averaged 208.91 ± 1.60 grams and 379 females averaged 191.81 ± 1.44 grams. The males had the higher coefficient of variability. The values are: 23.21 ± 0.57 for the males and 21.67 ± 0.56 for the females. The correlation of the weights of each individual chick at hatching at thirty-five days and at maturity (for the females only) is being determined. Data from 131 adult chickens show a coefficient of variability of 18.91 ± 0.82 for the gross body weight. The coefficients of variability for some of the organs are as follows: liver, 2.71 ± 0.11 ; feathers, 26.31 ± 0.13 , and blood, 2.02 ± 0.08 . The correlation of the weights of each of these organs with the gross body weights are: liver, $+ 0.62 \pm 0.04$; feathers, $+ 0.60 \pm 0.04$, and blood $+ 0.52 \pm 0.04$.

54. *The effects of diets varying in calorie value and in relative amounts of fat, sugar, and protein upon the growth of lymphoid tissue in kittens.* ROTHWELL LEFHOLZ, Department of Anatomy, University of Missouri. (Introduced by Eliot R. Clark.)

Settles found that a high fat—high calorie diet produces enlargement of lymphoid organs, particularly those in the alimentary canal. The present study was designed to find out whether the difference is due to the specific effect of the fat or to the calorie content. Five kittens were fed for five months on diets varying in percentages of fat, sugar and protein, and also in calories. The results indicate that the palatine and pharyngeal tonsils and the aggregated follicles of the intestine are regulated in size both by the calorie and by the specific fat content of the diet. A diet high in sugar or protein and also in calories produces in these organs a size nearly twice as great as a normal calorie diet, while if the excess calories are given in the form of fat the size is nearly three times as great. The number of solitary follicles varies enormously in different cats and is not regulated by the calorie or fat content of the diet. The size of the mesenteric lymph glands is greater in cats fed a diet high in fat than in those fed a high sugar or protein diet. Other lymphoid organs, spleen, hymus, cervical lymph glands, show no consistent response to variations in diet. There is evidence which indicates that there is a reciprocal relationship between the size of these organs, such that if one is unusually small others will be large, thus making the total amount of lymphoid tissue uniform.

55. *The effects of cell division upon the form of tetrakaidecahedral cells.* FREDERIC T. LEWIS, Harvard Medical School. (Lantern.)

A preliminary report of this study was made, unannounced, at the Philadelphia meeting of the Anatomists in 1921. The establishment of the tetrakaidecahedral form as typical for the cells in elder-pith has since been reported to the Zoologists (see abstract, *Anat. Rec.*, 1923, vol. 24, p. 378). This work will be briefly reviewed, and in addition the effects of cell division on fourteen-sided cells will be considered. Bisection of the cell, vertically or horizontally, produces cells with eleven surfaces or contacts with other cells, which are always potential facets. But if all the cells in a group divide transversely, the resulting half-cells continue to have fourteen surfaces or contacts. The quadrilaterals of the parent cell become hexagons in this process, and the hexagons produce quadrilaterals, restoring the tetrakaidecahedral form in a surprisingly simple manner. In some cases, as will be shown, cell division cuts off a third instead of a half of the original cell, or even a smaller proportion. The complete report of this study has been accepted for publication in the *Proceedings of the American Academy of Arts and Sciences*, vol. 58.

56. *The parthogenetic development of the egg in the ovary of the guinea-pig.* LEO LOEB, Washington University, School of Medicine. (Introduced by L. H. Weed.)

The development of trophoblast-like structures and of structures resembling embryos proper within the ovary of the guinea-pig. Conditions under which these structures were observed. The reaction of the host tissue; the fate of these structures

57. *In vitro cultures of mammalian embryos.* ALEXANDER A. MAXIMOW, University of Chicago, Department of Anatomy.

Explanation of young rabbit embryos shows three phenomena: 1) The usual 'histioid,' radial, 'uncontrolled' growth of tissues. 2) Individualization of explantate, epithelium covering its surface—an 'organoid,' controlled development. 3) Differentiation of anlagen.

The specificity of germ layers and tissues remains unaltered.

An embryonic shield (primitive groove) develops in three days into a complicated irregular structure with beating heart, blood vessels, medullary tube, and brain vesicles. Coincidentally the ectoderm and entoderm epithelium and mesenchyme may show histioid growth. The ectoplacental trophoblast usually proliferates; its ameboid giant-cells and syncytium invade and destroy the other parts; after six days only extensive multinucleated syncytial masses may remain.

Parts of ten- to twelve-day embryos first become spherical and are covered by epithelium, ectoderm, mesoderm, and entoderm always remaining sharply limited. Inside, mesenchyme, blood vessels, parts of intestine and medulla partly grow, partly degenerate. The periphery after some days may show histioid growth.

Coincidentally differentiation of anlagen manifests itself. The entoderm forms striated border; ectoderm shows cornification; anlage of mammary gland appears. Mesenchyme forms wandering cells and cartilage. The brain walls form folds, their cells form radial groups, neuroblasts develop axons, spongioblasts transform themselves into neuroglial wandering cells. Liver and pancreas anlagen develop extensively; after six days the former shows liver-cell rosettes with phagocytes, but without blood cells between them, the latter—large branching epithelial tubes. Both are

surrounded by growing mesenchyme with sympathetic ganglia and nerve plexuses. The gonad considerably increases in size.

58a. (A) *The iliac imprint of Bertaux and (B) digital impressions (foveae) in the supra-condylar foss of the femur.* A. W. MEYER, Stanford University Medical School.

I believe with Nicholas that this facet is postural in its origin, but I can not convince myself that it results from a sitting or squatting posture. Various considerations and especially the evidence obtained, especially from fresh preparations, seems to indicate quite clearly that it results from pressure of the iliac portion of the acetabular margin when lying on the side. A similar facet rarely is found on the posterior aspect of the femoral neck.

(B) Very rarely a small, shallow, but quite symmetrical depression is encountered in the supracondylar fossa of the femur. In two instances in which these foveae were present the knee-joints were markedly arthritic, and in all instances these foveae had the appearance of depressions which might have been made by digital pressure in a softened bone. They were located in the superior or cranial portion of the supracondylar fossa.

58b. *Evidences of attrition in the human body.* A. W. MEYER, Stanford University Medical School.

The walls of synovial bursae: articular capsules, discs, menisci and cartilages, and even tendons in contact with bones may be partly or wholly destroyed by friction in the course of years. It also seems highly probable that many cases of absence of the tendon of the long head of the biceps and deficiencies in the ventral wall of the articular capsule of the hip-joint, which have hitherto been regarded as congenital anomalies, usually are not such, but acquired defects in otherwise wholly normal articulations.

59. *The presence of atria in the human lung.* WILLIAM SNOW MILLER, University of Wisconsin.

The presence of atria in the human lung has been called in question by two recent writers on lung structure. That they failed to find them is not surprising, for the first author did not make a critical study of his preparations and the second author made use of methods which make their demonstrations exceedingly difficult unless one is well versed in lung structure and lung topography. It is now over thirty years since I began my study of lung structure and first described that division of the air spaces to which the name atrium was given. In the meantime reconstructions have been made from the lung of the cat, the dog (2), the ox, the child (2), and adult man (2). In every instance atria were present, and I can not but believe that any one following the methods made use of by the author will find no difficulty in demonstrating atria in mammalian lungs.

60. *On the relationship of the germinal epithelium to the position of the testis.* CARL R. MOORE, University of Chicago. (Introduced by F. R. Lillie.)

Testicular grafts (subcutaneous, intramuscular, or intraperitoneal) from normal male rats, castrated males, spayed females, normal females, and females having passed

through the periods of pregnancy, delivery, and suckling have been found to contain distinct seminiferous tubules and epididymis, after durations of one to six months. Epididymis tubules are usually normal, but spermatozoa have never been found in seminiferous tubules. Cells of the germinal epithelium may or may not be in active mitosis, but a normal germinal epithelium with spermatids or spermatozoa has not yet been obtained.

Some explanation of this is offered by experimental cryptorchidism. If guinea-pig's or rat's testicles are placed in the peritoneal cavity (through patent inguinal canals) without injury to the blood vessels, nerves, or vas deferens, degeneration of the germinal epithelium is noticeable after seven days; at fourteen days it is well advanced, and complete usually by twenty days (less extreme in rat than in guinea-pig). Within thirty to sixty days a few scattered, poorly stained cells (perhaps Sertoli cells) and reticulum characterize the tubules, and a very prominent interstitial cell mass gives a typical picture of a cryptorchid testis. Should the testis return to the scrotum after operation, it may or may not be normal, depending upon partially known conditions. It is understood, therefore, that the condition of the germinal epithelium is a function of the position of the testis in these animals; a scrotal testis is normal, an extrascrotal testis degenerate.

With this in mind, rat testes were grafted onto the walls of the scrotum. After six months some such grafts were found to contain normal seminiferous tubules with characteristic spermatozoa. This is the first case on record of spermatozoa having been differentiated in a mammalian testis graft.

61. *The distribution of blood vessels in the mammalian kidney.* DANIEL B. MAC-CALLUM, Hull Laboratory of Anatomy, University of Chicago. (Introduced by R. R. Bensley.)

The distribution of the arteries and their branches were studied in the rabbit, guinea-pig, rat, opossum, and dog by means of vital staining with janus green, followed by injection with carmine gelatine, vital staining, without subsequent injection, and injection with colored suspensions of various sorts. Huber's alkanin celloidin mass was also employed. The result of these studies is a complete confirmation of the results obtained by Huber with regard to the origin of the arteriae rectae, and the general character of the circulatory apparatus of the mammalian kidney. These results may be summarized as follows. All of the branches of the renal artery entering the renal substance in the mammals mentioned terminate in glomeruli. The direct branches of vasa afferentia described by Golubew do not exist. The direct communications between arteries and veins described by Golubew do not exist. The retia mirabilia described by Golubew at the level of the arciform arteries, are probably horizontally directed showers of arteriae rectae spuriae. There are in the mammals studied no arteriae rectae verae. All straight arteries of the medullas are derived from efferents of glomeruli. The exceptions to these results are exceedingly rare and do not constitute a factor which requires consideration in functional discussions. They are as follows: Some branches of the renal artery destined for the capsule plunge through the kidney substance and on their way to the capsule give off afferents to glomeruli. An occasional interlobular artery is continued past the origin of the last afferent out to the capsule. Some afferents terminate in small glomeruli which may consist of a single U-shaped capillary loop.

The efferents of such small glomeruli are of large size and are distributed to neighboring tubules. The majority of apparent arteria rectae verae resolve themselves under the binocular into branches of other vessels passing over or under the arteries from which they appear to arise. A great deal of error has been introduced into the literature of this subject, owing to the difficulty of distinguishing, in injections of one color, between arteries and veins.

62. *On the growth of the larynx, trachea, bronchi and lungs during the fetal and neonatal periods, with a consideration of their vertebral levels through infancy and childhood.* G. J. NOBACK, Medical College of Virginia. (Lantern.)

The dimensions and levels of the several organs were obtained, after dissection, in situ. One hundred two fetuses and new-born infants and several children were utilized as well as the data as recorded in the literature. The writer's data were treated statistically and graphically. All the curves of the dimensions (height or length, width, anterior-posterior diameter) resulting from plotting the measurements were of the Straight-Line type and may be expressed by the following type of formula, $Y = aX \pm b$. Y , is the dimension studied; X , body length; a and b , empirically determined constants. Thus it is determined that the growth of these parts of the respiratory system undergoes a steady and constant growth during fetal life and the period of the new-born. It bears the same relation to body lengths as do most of the external body dimensions as recorded by Calkins and as that of the cerebrum as determined by Dunn. The cranio-caudal direction of development is emphasized by the findings in regard to the relation of these organs to the vertebral column. There is a general descent beginning with the early fetus and through the neonatal period. The descent is least in the case of the upper limit of the larynx and most at its lower limit and for the tracheal bifurcation. The apices of the lungs undergo less descent than the inferior margins of the lungs. The data in the literature indicates that the levels in the new-born are maintained through infancy and that in early childhood there begins a descent to levels as found in the adult.

63. *The carpus of Eryops and the structure of the primitive chiropterygium.* G. K. NOBLE, The American Museum of Natural History. (Introduced by H. D. Senior.) (Lantern.)

Reexamination of the most primitive carpus known that of *Eryops megacephalus*, has demonstrated that only four, not five digits were present. The first carpal ray carried a well-developed prepollex. All known Amphibia, recent or fossil, possess only four digits in the manus, but embryological and indirect palaeontological evidence has allowed us to infer that the most primitive Amphibia had a prepollex, five digits and a postminimus in the hand; a prehallux, five digits and a postminimus in the foot. The primitive chiropterygium was therefore at least seven-rayed in both the manus and pes. The carpus (as well as the tarsus) consists of two moieties; the radius (tibia) moiety, embracing the prepollex (prehallux) and carpal elements, forming the first preaxial ray, and the ulna (fibula) moiety, including the digits and their carpals, converging toward the ulna (fibula). The distinctness of these two moieties is further demonstrated by the subdivisions of the carpal (or tarsal) musculature. The carpus of *Eryops* is readily comparable with the pectoral fin of the rhipidistian crossopterygians. The distinctness of the two primary series of carpal

elements is clear in these forms. The carpus of *Eryops* and the primitive chiropterygium possessed three medialis and one centrale, as well as the radiale, intermedium, ulnare, and the carpalia. The phalangeal formula of *Eryops* was only 2-2-3-2 in the manus. These observations were made in collaboration with Dr. W. K. Gregory and R. W. Miner.

64. *Primitive pores and nerve sacs of the cartilaginous ganoids.* H. W. NORRIS, Department of Zoology, Grinnell College.

That the 'primitive pores' of the paddlefish and the 'nerve sacs' of the sturgeons are homologous sense organs is shown by their structure, their topographical distribution, and their innervation. They are not taste organs, but lateral-line organs, for in *Polyodon* the visceral sensory centers of the brain are meager in their development and the lateral-line centers extraordinarily large (Black, '16-'17), and the visceral sensory nerve supply to the rostrum, upon which enormous numbers of these sense organs occur, is vestigial. The buccal nerve (lateral line), however, whose terminations are related to these sense organs on the rostrum, is unusually large.

65. *The brain of Coenolestes obscurus.* JEANNETTE BROWN OBENCHAIN, (Introduced by C. Judson Herrick.) Department of Anatomy, University of Chicago.

Material. A transverse iron-haematoxylin series of the brain of an adult female of this lowly South American marsupial. Herrick has figured the surface of this brain in Osgood's Monograph on *Coenolestes* ('21).

The mesial aspect, as reconstructed, resembles that of lowly Australian polyprotodont marsupials rather than *Didelphys* or the diprotodonts. The 'aberrant bundle' of the anterior commissure, considered by Elliot Smith a diagnostic character of diprotodont brains, is absent. This is of interest in view of Osgood's recent classification of *Coenolestes* as a diprotodont of generalized type, on the basis of dental and other features.

The enormous amygdaloid complex, occupying the entire base of the hemisphere, is superficial and sharply separated from the pyriform cortex, save posteriorly. An incomplete amygdaloid fissure marks the line of junction externally. The hippocampus fuses posteriorly with the pyriform lobe; that is, it does not curve far forward into the temporal pole. The gyrus dentatus is superficial throughout. The tuberculum olfactorium exhibits a spectacular development of 'islands' and convoluted 'cortical' cell-layer.

66. *On the factors in testicular degeneration following ligation or vasectomy.* ROBERT OSLUND, (Introduced by F. R. Lillie.) University of Chicago.

It is generally believed that ligation and resection of the vas deferens results in degeneration of the germinal epithelium and hypertrophy of the interstitial cells. Observations from approximately 125 experiments on rats and guinea-pigs fail to confirm this idea. Ligation and resection have been carried out on rats from thirty days of age to adults, and on guinea-pigs from twelve days of age to adults. Histological studies on testes from one to ten months after operation show that such testes are to all appearances normal; spermatozoa in abundance are present. Results of unilateral vasectomy, bilateral vasectomy, unilateral vasectomy with removal of the opposite testicle, bilateral vasectomy with one testis forcibly retained within

the abdomen, unilateral vasectomy with the normal testis in the abdomen, as well as forcible retention of both testes without ligation or vasectomy are in accord with the following explanation: In any operation involving retention of the testis in the peritoneal cavity (with or without vasectomy) degeneration of the germinal epithelium follows. This degeneration, however, is not the result of the ligation or resection, as such procedures fail to produce degeneration, provided the testis remains in the scrotum; if normal testes are retained for but a short time in the peritoneal cavity, degeneration is in evidence. Associated with varying stages of degeneration of the germinal epithelium there appears to be a parallelism of interstitial cell hypertrophy.

67a. *The Mammillary Peduncle Marchi Method.* J. W. PAPEZ, Cornell University Medical College, Ithaca. (Lantern.)

Lesions of the mammillary body and tegmentum were produced experimentally in rats by inserting a hooked instrument into the pharynx and puncturing the basiphrenoid, hypophysis, and dura. A unilateral injury of the mammillary peduncle near its pontine end produces within two weeks a complete degeneration of this bundle in a cephalic direction. This demonstrates that the bundle is an ascending one and ends chiefly in the lateral mammillary nucleus. This is contrary to the results obtained from mammillary lesions in the dog by Schipoff in Bechterew's laboratory. It, however, accords with Cahal's ('03) description based on Golgi preparation in the mouse in which the details of termination are shown. No fibers arose from substantia nigra as Ganser ('81) thought. The degenerations in the rat showed an upward continuation of a small number of fibers into lateral nucleus of the tuber cinereum, but there was no connection with the zona incerta or column of the fornix as Honeggar ('90) believed. The degenerations in rats contradict the view of Kölliker ('96) and Dejerine ('01), according to whom the tract arises from the lateral mammillary nucleus and descends. In a direct lesion of the mammillary body in a rat the bundle did not degenerate downward. No evidence concerning the origin of the bundle is contributed here.

67b. *The thalamic end of the medial lemniscus. Marchi method.* J. W. PAPEZ, Cornell University Medical College, Ithaca. (Lantern.)

When the medial lemniscus is cut in the tegmental region in the rat, Marchi preparations show that this bundle has two sets of endings in the Thalamus. The principal terminals course along and in the ventral surface of the ventral and lateral nucleus of the thalamus forming a narrow crescentic lemniscal stratum. The degeneration of these terminals is limited almost exclusively to this nucleus. No fibers enter the main portion of the zona incerta; none were traced to the striatum, or cortex; a very doubtful few were observed in the substantia nigra and posterior commissure. The other terminals arise at right angles from the medial lemniscus in the caudal limits of the thalamus opposite the posterior commissure. They pass dorsally into the posterior nucleus of the thalamus in and against the course of the numerous thalamocortical fibers of this region. These fibers probably correspond to the group of dorsally directed collaterals described by Cahal ('00) in the mouse. In the adult rat these degenerated fibers appear to be coarser and not so numerous as those figured by Cahal.

67c. *The rubrospinal tract, Marchi method.* J. W. PAPEZ, Cornell University Medical College, Ithaca. (Lantern.)

Following a tegmental lesion in a rat in which the nucleus ruber of one side and the contralateral rubrospinal tract were injured. There was after seven days a complete bilateral descending degeneration of the rubrospinal tracts. From the injured nucleus the rubrospinal tract passed through the ventral decussation of Forel to the opposite side and descended medial to the lateral lemniscus, ventral to the trigeminus, facial nucleus, etc., in the exact position described in various mammals by other authors (v. Monakow, '83; Held, '90; Probst, '99; Rothmann, '00; Pavlow, '00; Van Gehuchten, '01; Rasmussen, '22, and others). This confirms Held's ('90) myelination observations on this bundle in the rat. The opposite bundle degenerated downwards below the lesion, but no retrograde degeneration towards its nucleus of origin occurred, such as Van Gehuchten found in rabbits twenty-five to thirty days after the lesion. However, the cells of the nucleus ruber appear to have undergone all but complete chromatolysis as observed by Van Gehuchten in the rabbit six and one half days after injury of the tract.

68. *Myeloid metaplasia in the omentum of normal rabbits.* EMILY PAYNE, (Introduced by Hal Downey.) University of Minnesota.

Several previous investigators have noted the occasional occurrence of developing granular leucocytes in the omentum of young and experimental animals. A very few have likewise noted the presence of developing red cells in the omentum of newborn animals and of human embryos. Up till now, however, there has appeared no detailed account of the cells in the omentum from a histiogenetic standpoint.

A recent investigation by the writer on the omentum of the rabbit reveals the fact that in young animals myeloid metaplasia, especially the development of pseudo-eosinophils, is of such frequent occurrence as to be almost characteristic of the organ. In the omentum of adult rabbits, granulopoiesis is much less frequent; nevertheless, it may be found in apparently normal untreated animals. Developing granulocytes may occur in large patches or as small groups of cells scattered throughout the tissue. Developing reds, on the contrary, invariably occur in characteristic nests. An important relationship appears to exist between the centers of myeloid metaplasia and the degeneration of vessels.

69. *A note on the mechanics of the drum-membrane-malleus attachment.* A. G. POHLMAN, St. Louis University. (Lantern.)

Further investigation on the problem of the mechanics of sound transmission required the development of special apparatus for testing the acuity of hearing. This apparatus was designed and set up by the Riverbank Laboratory through the courtesy of Col. George Fabyan. Dr. F. W. Kranz will demonstrate some of these appliances at this meeting.

Kranz and the writer worked out certain features in reference to the effect of plus and minus pressures in the air of the external auditory canal on acuity of hearing. In a general way we found that a minus pressure equal to a water pressure of 10 cm. presented optimum conditions. The details of the acuity curve for the tone range, the quantitative effects of plus and minus pressures in high and low pitches, and the mechanical interpretation of the pressure effects on the connection between the drum membrane and malleus will be discussed.

70. *The tectospinal fasciculus in the cat.* A. T. RASMUSSEN, University of Minnesota. (Lantern.)

The tectospinal fasciculus (descending fibers from the tectum of the midbrain) as found in the higher mammals has been variously described as to the completeness of its decussation, its termination in the spinal cord, and its contribution to the medial longitudinal bundle. There are many disagreements on these points in the same species, e.g., the cat. The origin, course, and termination of these fibers in a dozen cats have been tested by the Marchi method after extirpation of various parts of the midbrain. The results warrant the following conclusions: 1) the fibers all cross the midline (decussating mostly in the dorsal tegmental decussation, but also to some extent dorsal to the aqueduct); 2) the degeneration on the same side as the lesion, which has been taken as evidence of direct fibers, is due to fibers from the unoperated side having crossed dorsal to the aqueduct and been cut after crossing; 3) tectospinal fibers cannot be followed by the Marchi method below the seventh cervical segment; 4) there is no evidence that fibers from cells in the superior and inferior colliculi descend in the medial longitudinal fasciculus. The probable significance of the tectospinal fasciculus as a visual reflex pathway will be discussed.

71. *The comparison of fish and tetrapod limb muscles.* A. S. ROMER, Bellevue Hospital Medical College.

Previous attempts to homologize the limb musculature of fish and land vertebrates have been handicapped by our lack of knowledge of both the osteology and the myology of the primitive tetrapods. Recent palaeontological work, however, has thrown some light upon the nature of the transitional stages, in the shoulder region especially; the primitive shoulder musculature has been described by Watson, the writer, and Miner.

The musculature of the primitive tetrapod limb may be resolved into two groups, comparable with the two opposed muscle masses of the fish fin: 1) dorsomedial adductors and, 2) ventrolateral abductors. 1) A dorsal mass which includes the equivalents of latissimus dorsi, teres major, subscapularis, triceps, and of the muscles of the extensor aspect of the arm, forearm, and hand. 2) A ventral mass, which includes the equivalents of the teres minor, infraspinatus, supraspinatus, deltoideus, pectoralis, coracobrachialis, biceps, brachialis, and of the muscles of the flexor surface of the arm, forearm, and hand. (In man several of these muscles are dorsal in position, but all of them, with the exception of the deltoid, are ventral in existing reptiles or amphibians.) A triple method of innervation of the ventral group is found in both fish and tetrapods through equivalents of, a) circumflex, b) suprascapular, c) musculocutaneous, median and ulnar nerves. The pelvic limb may be treated in like fashion, as dorsal and ventral groups arising from dorsomedian and ventrolateral surfaces of the primitive pubo-ischiatic plate. The tetrapod ilium has taken over most of the dorsal muscular origins.

72. *New empirical formulae for determining the age of the human fetus.* RICHARD E. SCAMMON and L. A. CALKINS, Department of Anatomy, University of Minnesota. (Lantern.)

Simple and accurate formulae for the determination of the probable age of the fetus from its external dimensions are of great importance in the study of human

growth, and none of the four published conventions fully meet the requirements. An extensive study of the available data on the relation between body length and age in prenatal life shows a large group of observations (gathered independently by four modern investigators) which were selected for use because of their consistency throughout the period. We have developed several simple algebraic expressions for this material. In general these formulae express age in fetal or lunar months in terms of the first and second powers of the total body length modified by constants. Their application involves simple arithmetic only. These empirical formulae were developed primarily for the expression of the relation of total body length to the menstrual age, but they are easily modified to express the relations of weight, cohabitation age, and crown-rump length.

73. *The postnatal growth in weight of the human brain and its parts and of the spinal cord.* RICHARD E. SCAMMON and HALBERT L. DUNN, Department of Anatomy, University of Minnesota. (Lantern.)

Although most curves of the growth of human organs and parts between birth and maturity are of a highly complicated nature, those of the growth in weight of the human brain, when plotted against the age in years, are hyperboloid in form and may be represented with some accuracy with simple empirical formulae. Similar formulae have also been developed for expressing the postnatal growth of the cerebrum, cerebellum, brain stem, and spinal cord. The sex differences in weight of the brain as a whole and its larger divisions (when calculated in relation to age) are sufficiently great to permit the development of separate formulae for males and females. The growths in weight of the brain and its major parts during most of the first year may be represented fairly well by straight lines and correspondingly simple empirical formulae. Formulae have also been computed for expressing the relation between brain weight and body length in the postnatal developmental period, but these are less satisfactory than those based on age, probably because of the incomplete nature of the data available for study. The formulae mentioned above have been used as a basis for calculating the relative growth of the brain and the velocity of growth at various periods.

74. *A microchemical study of the adrenal body.* H. SHEPPARD, School of Medicine and Emory University.

By means of certain microchemical methods it is possible to demonstrate that the cortex of the adrenal body functions as a complex glandular organ of internal secretion. All of the parenchymal cells of the cortex are heavily loaded with a lipid substance in a normal condition, but when the organism is subjected to toxins, poisons, fatigue, or any condition which will produce an injury to the tissue, and especially the red-blood cells, this lipid substance is rapidly used up and the cortex can be rendered practically free of its fatty material. Cholesterol esters fill the cells of the zona glomerulosa and the outer part of the zona fasciculata. That part of the cortex which borders upon the medulla contains choline.

By means of a specific adrenalin stain in combination with scharloch R, it can be shown that there is no lipid material in the medulla, but it is easy to show that adrenalin fills the entire medulla as well as the blood-vessels. Further, the adrenalin material is not limited to the medulla alone, but extends up in the cortex to the point

where it is being produced from the various lipoids, possibly by an enzyme action. The breaking up of the lipid bodies in the cells of the cortex into the small bodies which go to form adrenalin can be observed by proper fixation and staining so that a complete picture of the process is seen in a single section.

75a. The response of the hypophysectomized tadpole to the intraperitoneal injection of the various lobes and colloid of the bovine hypophysis. PHILIP E. SMITH and IRENE P. SMITH, Anatomical Laboratory, University of California.

The intraperitoneal injection in the hypophysectomized tadpole of the various lobes and the colloid of the bovine pituitary brings about definite and clearly defined responses. The anterior lobe elaborates a principle, or principles, which when injected restore to normal the individual suffering from the four rather sharply defined disturbances which result from the early ablation of the buccal hypophysis, namely: 1) a pigmentary upset resulting in albinism, 2) a retardation in growth, 3) a failure of the thyroid, parathyroid, and interrenal organ to develop normally, and 4) a large and persistent fat-organ which would appear to indicate a disturbance in fat metabolism. The process of restoration may pass the normal mark and gigantism, an enlarged, overactive thyroid, and an unusual depth of pigmentation may result.

Injections of the pars intermedia (probably slightly contaminated with pars neuralis) cause a return to normal color and appear to bring about a normal size of the fat-organ. No response in the thyroid results, legs do not grow, and the specimens do not metamorphose. There were indications of a slight growth response. Injections of the posterior lobe cause a definite, though lesser pigmentary response than that elicited by the pars intermedia, and the characteristic larger fat-organ is not found. No leg growth or metamorphic changes take place. Colloid appears entirely inert save for a moderate pigmentary response which is evoked by its injection. It appears from this and other physiological tests to be a waste production.

75b. The topographical separation in the bovine anterior hypophysis of the principle reacting with the endocrine system from that controlling general body growth, with suggestions as to the cell types elaborating these encretions. PHILIP E. SMITH and IRENE P. SMITH, Anatomical Laboratory, University of California.

If the bovine pituitary be opened by a medial sagittal cut, a darker central area extending through the longitudinal axis will usually be seen which is readily distinguishable from the surrounding light, lipid-like area. Sections of glands showing a well-marked gross separation reveal the fact that the central area is composed of chromophobes and basophiles, the outer area of eosinophiles and chromophobes, among which are mingled scattered basophiles.

Hypophysectomized tadpoles injected (1 part saline to 3 parts gland) with these two parts react quite differently. Those receiving the central part grow more slowly and advance towards metamorphosis more rapidly than those receiving the outer portion. The thyroid is usually hyperplastic, the specimens exhibiting some of the characteristics of thyroid-treated specimens. Those receiving the outer portion grow unusually large, have a normal body—leg proportion and their thyroids are not hyperplastic.

A perfect separation of the gland for purposes of injection into parts containing only one of the chromophile cell types cannot usually be made, since basophiles are scattered throughout the gland, and the central part is not infrequently invaded by eosinophiles. However, it can be stated that the majority of the cells present in that portion giving a predominant thyroid response are basophiles and chromophobes. The evidence here indicates for the first time that there is a separation of the principle stimulating growth from that reacting with the endocrine system, and that each of these principles is elaborated by a specific cell type.

76. *The response of the Colorado axolotyl to the intraperitoneal injection of the thyroid, anterior hypophysis, and combined injections of these two substances.* PHILIP E. SMITH, Anatomical Laboratory, University of California.

As is well known, the Colorado axolotyl, although it may remain neotoneous in its native habitat, will promptly metamorphose during the summer months when taken to a lower altitude. Injections of anterior-lobe substance prolong the larval period under the conditions, causing also an increased rate of growth and pigmentation. The action of anterior lobe seems here to be opposed to thyroid activity. This was further tested by combined thyroid-anterior-lobe injections as suggested by Doctor Evans. Two groups of specimens were given identical thyroid dosages, one group receiving in addition anterior lobe. Those receiving thyroid only, metamorphosed promptly, the others metamorphosed very slowly or not at all.

This might be considered as indicating a neutralization, in a chemical sense, or a direct repressing action of the anterior lobe on the thyroid. Such we believe not to be the case. It seems that two factors influence behavior here: one, characterized by Swingle and Uhlenhuth as catabolic, is controlled by the thyroid; the other, constructive (growth), is controlled by the anterior hypophysis. As long as the constructive factor predominates, whether due to a favorable larval habitat or to introduced anterior hypophyseal substance, the catabolic phase of metabolism does not express itself.

The response elicited by anterior-lobe injections appears always constructive. General body growth is obviously so. The repair of the atrophic thyroid in the hypophysectomized tadpole following such injection also appears as a constructive response, although it leads the repaired gland to function, thus secondarily inducing activity of a catabolic nature.

77. *The blood supply of the labyrinth in birds.* CARL F. SNAPP, (Introduced by R. R. Bensley) Hull Laboratory of Anatomy, University of Chicago.

The blood supply of the vestibular labyrinth is through the labyrinthine artery, which enters through the meatus acusticus internus, and at once divides into two main divisions which supply the semicircular canals, utricle, and saccule. A smaller artery, possibly a branch of the labyrinthine, enters on the concave side of the cochlea to supply that structure. The venous system is divided into two parts in the chicken and pigeon, both passing along the aqueductus cochleae, while in the duck and goose these are united into one large system which drains through a large vein passing out along the aqueductus cochleae. In all four birds examined a communication exists between the perilymph spaces of the posterior and horizontal canals at the point where they cross, but there is no such communication between the endolymphatic spaces.

78. *The age-order of epiphyseal union.* PAUL H. STEVENSON, Anatomical Laboratory of Western Reserve University, and Peking Union Medical College, Peking, China. (Lantern.)

Recognizing the dominant importance of the phenomenon of diaphyseal epiphyseal union in questions concerned with age determination in the skeleton, the present study seeks to establish the accuracy of our knowledge of the time elements concerned upon a more authoritative basis than has hitherto been done. The results to be presented are based upon a detailed study of the various stages of epiphyseal union observed in a series of over one hundred definitely age-known skeletons, all within the age period of actual epiphyseal union. The authority of such a study depends upon the number of skeletons studied, the unquestioned normality of the material used, and the definiteness of the age data accompanying each individual skeleton. All material failing in any way to satisfy any one of these three conditions has been rigidly excluded from the series under observation. Two phases of the subject are dealt with, 1) the time of union of the various epiphyses and, 2) the sequence of these unions. An effort is made to correlate the time data secured and to express it in a form directly applicable to the determination of age in the case of skeletal material of unknown age.

79. *Germinal defect as a cause of abnormal development.* GEORGE L. STREETER, Department of Embryology, Carnegie Institution of Washington.

The investigators who have studied pathological human embryos have laid stress on environment and faulty implantation as causes of defective development. Those working with other animal forms, however, have shown that the factors resulting in defective embryos are to be traced back to the ova in their earliest stages before implantation. The case that the writer presents would indicate that our future experience will show that germinal defects play a large part in the pathology of the human embryo. A description will be given of a defective fetus and placenta, of a type very commonly met with in abortions, where the defect must be regarded as of germinal origin. The environment as a factor is ruled out by the circumstance that it was a case of double-ovum pregnancy, in which the other ovum went through to term as a normal living baby. The normal and abnormal placentae are directly contiguous and so must be regarded as exposed to essentially the same surface. At their junction there is an abrupt demarcation, on one side of which the villi are in an advanced stage of fibrinoid degeneration, while on the other they are entirely normal.

80. *Further observations on the order, time, and rate of ossification in the chick, duck, and goose.* R. M. STRONG, Department of Anatomy, Loyola University School of Medicine.

The bones of the skull begin to ossify in the duck, mostly two to three days later than in the chick. In the goose, nine out of nineteen skull bones compared, began to ossify one day later than in the duck, and nine at about the same time. First ossification in the vomer was observed in the chick and duck on the thirteenth day of incubation, and on the fourteenth day in the goose. It had begun on the eighth day in the mandible and in the nasal bone. The first ossification in the clavicle was found on the eighth day in the chick, the eleventh in the duck, and the twelfth in the goose. The long bones of the arm and leg were one day earlier in the chick, and two days earlier in the duck and goose. Other details will be given in tabular form.

81. *Parallelism of variation in a mammalian series.* R. J. TERRY, Washington University.

There is now abundant evidence showing that the variation of the humerus in man known as the supracondyloid process presents itself in several definite forms which are regarded as indicating different degrees of development. The forms recognized are: a bony bar extending from the shaft to the medial epicondyle; a spinous process of the shaft; a tubercle, a ridge, and an irregular rough spot. The relative frequency of these forms has been determined in certain white races and American Indians. If the underlying factors in the production of a supracondyloid process are the same in man and in those mammals in which a supracondyloid bar is a normal feature of the humerus, then it might be expected that variations of the bar would in part repeat those characteristic of the human humerus. This is apparently true as determined by the examination of a considerable series of mammalian fore-limb bones.

82. *The development of body movements in relation to the maintenance of the visual field.*

HENRY C. TRACY, University of Kansas.

The order of development of the different phases of the reaction to rotation in toad-fish larvae (*Opsanus tau*) is as follows: 1) Slow phase of nystagmus during rotation; slow roll of eyes back to resting position on stopping rotation (11 mm.). 2) Compensatory coil of body (11.5 mm.). 3) Back stroke of body on stopping rotation; eye comes to rest through series of oscillations (12 mm.). 4) Quick phase of nystagmus (15.5 mm.). 5) Active swimming opposite to direction of rotation (young adults).

Compensatory reaction of body consists of a coil to left on clockwise rotation, to right on contra-clockwise rotation. Position of fish with reference to rotation does not influence the reaction. If the larva is inverted, compensation is as above, but involves opposite side of the body. The eye reacts to a smaller angle of rotation than the body (5° or less in older larvae). Rotation reflex and 'Lyon's reflex' are 'allied reflexes' holding field of vision constant while the head swings from side to side during swimming. Many rotation movements apparently do not produce disequilibrium; all tend to dislocate the visual field. Animals usually move in space by asymmetrical movements which cause rotary motions of head. Dislocation of visual field is accurately compensated for by eye and body reflexes. Character of these compensatory movements, order of development, delicacy of reaction, alliance with other reflexes indicate, that their chief significance is in maintaining the visual field constant during the varied movements of the animal in its environment.

83. *The anthropoid strain in the human pubic symphysis.* T. WINGATE TODD, Medical Department, Western Reserve University.

1. The human pubic symphysis presents a Eutherian metamorphosis in modified form as a result of the permanent separation of the two pubic bones. 2. Pubic metamorphosis in the giant Anthropoids is the connecting link between the human and the more typical Eutherian form. The epiphyseal remnants of the human symphysis, namely, ventral rampart, upper extremity, and to a less and more variable extent the lower extremity, are the homologues of median bar and upper and lower nodules of typical Eutherian metamorphosis. 3. Among human symphyses, both

White and Negro, there exist two well-defined types of metamorphosis. The first has the epiphysical remnants obtrusively developed with consequent modification and even inversion of the phase-age relationship as originally enunciated. The second shows little or no independent epiphysial formation and is the modal type in both human stocks. The first of these types is clearly related to one form of metamorphosis as shown in the giant Anthropoids, and therefore is called the anthropoid strain. The second is named the regressive type of human metamorphosis. Between these two modes there is an intermediate type of metamorphosis which serves to link the other two. 4. The three types occur in Whites as in Negroes, but are more strikingly distinguished in the latter. There is no great difference in relative frequency. 5. Examples of both the anthropoid strain and the regressive type may be accelerated or retarded in time relationship, and such examples differ in their exact features according to the type to which they belong. In addition, actual retrogressive examples of each type occur; these often present anomalies of outline or of surface contour in addition to the retrogressive features of the symphysial face. 6. In estimating age by the symphysis one must be cautious in evaluation of the several symphysial features, those of the pubic bone itself being much more reliable than the various epiphysial remnants. If the type is clearly distinguished, however, these remnants are of distinct help in coming to a correct conclusion.

84a. *On the nature of mitochondria. VI. A comparative study of the fragility of bacteria and mitochondria.* IVAN E. WALLIN, University of Colorado.

An analysis is made of the variation of fragility in bacteria, as well as an analysis of the character of fragility in mitochondria. The relationship of fragility to staining reactions is discussed.

84b. *On the nature of mitochondria. VII. The independent growth of mitochondria in artificial culture media.* IVAN E. WALLIN, University of Colorado.

A discussion of evidence in bacteriological literature of bacterial and mitochondrial growths in artificial culture media after inoculation with animal tissues. The description of methods used by the author in the production of independent growth of mitochondria in artificial culture media. Some characteristics of the organisms grown are described. The fundamental significance of mitochondria is briefly discussed.

85. *The anatomy of the seminal vesicles of the guinea-pig.* ARCHIBALD W. WARNOCK, Stanford University Medical School. (Introduced by A. W. Meyer.)

Measurements on the seminal vesicles of twenty-one adult pigs showed the existence of a remarkable bilateral symmetry in the mass of the vesicles. Each vesicle formed approximately 0.2 per cent of the body weight. However, a pig twice as heavy may have vesicles only half as large. Except near the base, spermatozoa were never found present in the vesicles, thus confirming Minot in the main. Diverticula were found most common in immature animals in which the free extremities of the vesicles frequently were bifurcated. Plicae circulares were found in all portions except the proximal half centimeter. They were found developed best in active, mature animals, and sometimes were complex. The epithelium and also the submucosa differs decidedly in the turgid and resting, as compared with the active

state of the vesicles. The longitudinal musculature is inconspicuous in distended vesicles except near the base. A transverse zone of more or less oblique fibers exists between the dorsal band of longitudinal fibers and the inner circular layer. No solvent for the secretion of the vesicles was found among the ordinary laboratory solvents. It is composed of a homogeneous substance, present in both depleted and turgid vesicles, and a finely granular material found only in the depleted state.

86. *A comparison of the nucleic acid content of various tissues.* BEATRICE WHITESIDE, Washington University.

In the course of an investigation on situs inversus I had occasion to extract nucleic acid from mollusks. The oyster and periwinkle were used and the acid obtained from the whole animal by Neumann's method. The product possessed the same physical and chemical properties as thymus nucleic acid. The quantity of nucleic acid in various mammalian tissues was compared with the amount of nuclear material present. The proportion was shown to be direct.

87. *Unmyelinated nerve fibers of the dorsal root.* W. F. WINDLE, Northwestern University Medical School. (Introduced by L. B. Arey.)

Ranson and others found that the small cells of the spinal ganglia give rise to unmyelinated fibers which divide dichotomously, sending one branch toward the cord and one peripherally. He was able to trace them for long distances in the spinal nerves, but, due to difficulties of technique, was not able to demonstrate them in the dorsal roots except as they enter the root from the ganglion and as they leave the root to enter the cord. Miss Parsons, using the same technical methods, concluded that these fibers were neuroglia, but Miss Wilson showed that neuroglia fibers are not present in the roots and ganglia. In a recent paper, Langley finds practically no unmyelinated fibers in the dorsal roots. He used the osmic acid method altogether.

We have obtained good pyridine-silver preparations of the dorsal roots by imbedding them in cord before fixation. Osmic-acid preparations were also studied. Unmyelinated fibers are not visible in the osmic-acid preparations, but stand out sharply in all parts of the root in the pyridine-silver material. They are even more numerous than the myelinated fibers and vary in diameter from one-half to a little over 1 μ . The question of the presence of these unmyelinated fibers is of considerable physiological importance because there is good evidence that they mediate painful afferent impulses.

DEMONSTRATIONS

1. *The red blood cells of the new-born albino rat.* W. H. ADDISON and M. W. RICHTER, University of Pennsylvania.
2. a) *The growth rate of roosters and hens from which the pineal glands were removed when newly hatched.* b) *The growth rate of control hens and roosters.* c) *The weights of the testes and combs of the operated and control roosters.* J. A. BADERTSCHER, Indiana University School of Medicine.
3. *Sections and models of human embryos from 2 to 3½ mm. in length.* G. W. BARTELMIZ, University of Chicago.
4. a) *Peritoneal sac and retroperitoneum.* b) *Mesomuscles.* O. V. BATSON, College of Medicine, Cincinnati University.

5. a) *Curves of actual size increment.* b) *Curves of percentage increment.* c) *Curves of alternation in growth.* R. B. BEAN, University of Virginia.
6. *Preparation of pancreas, and thyroid glands to illustrate various secretory and experimental conditions.* R. R. BENSLEY, University of Chicago.
7. *Regulatory adjustments in nerves and arteries of a dicephalous pig embryo.* MABEL BISHOP, University of Chicago. (Introduced by C. Judson Herrick.)
8. *Field graphs and curves illustrating the relative and absolute growth of the vertebral column and its various segments, and the individual vertebrae in the fetal period.* L. A. CALKINS, Department of Anatomy, University of Minnesota.
9. *The reaction of living cells in the tadpole's tail to injected bacteria.* E. R. CLARK, University of Georgia.
10. a) *Histological preparations showing nerve cells in the floor plate of Amblystoma.* b) *Projections and graphs showing relative rates of proliferation and differentiation of cells in the central nervous system of Amblystoma as correlated with physiological stages of development.* G. E. COGHILL, University of Kansas.
11. *Prints and tracings of epidermal ridge configurations: a) in various developmental defects and, b) illustrating individual variation of the planta, proximal to the ball region.* HAROLD CUMMINS, The Tulane University of Louisiana.
12. *The form of the shaft in several types of hair.* C. H. DANFORTH, Department of Anatomy, Stanford University School of Medicine.
13. *Models of Norway and albino rat brains.* Prepared by Dr. C. H. Heuser. H. H. DONALDSON, The Wistar Institute of Anatomy and Biology.
14. *Microscopic preparations of leukemic blood and of normal blood-forming organs illustrating the occurrence and relationships of the so-called 'myeloblast.'* HAL DOWNEY, Department of Animal Biology, University of Minnesota.
15. a) *Figures, graphs and tables illustrating the statics of the arch of the human foot when subjected to loads.* b) *Tracings, graphs and tables illustrating the growth of the integral parts of the cerebral hemispheres from the third fetal month to adult life.* HALBERT L. DUNN, Department of Anatomy, University of Minnesota.
16. *Graphs and tables illustrating the neonatal changes in the number of palpable lymph nodes in the human infant.* LOUIS DUNN and HALBERT L. DUNN, Department of Anatomy, University of Minnesota and Asbury Hospital, Minneapolis.
17. *The action of neutral red and Janus green as histological stains.* HERVEY S. FARIS, University of Kansas. (Introduced by G. E. Coghill.)
18. *Preparations of striated muscle degenerating as a result of nerve-section.* THEOPHIL GRAUER, University of Chicago. (Introduced by R. R. Bensley.)
19. *Models illustrating functionally defined regions of adult and larval brains of Amblystoma.* C. JUDSON HERRICK, Anatomical Laboratory, University of Chicago.
20. *Specimens illustrating the histology of the parathyroid gland of the albino rat.* MARGARET M. HOSKINS and SIMON B. CHANDLER, University of Arkansas, Medical School.
21. *The myenteric plexus before and after degenerative section of the vagus and splanchnic nerves.* S. E. JOHNSON, Northwestern University Medical School.
22. *Preparations of brains of animals and of human fetuses to illustrate the evolution of the corpus striatum and the amygdaloid complex.* J. B. JOHNSTON, University of Minnesota.

23. *The morphology of the Purkinje fibers in different parts of the atrioventricular bundle of the calf's heart.* P. N. JOHNSTONE and F. H. WAKEFIELD, University of Missouri and University of Georgia. (Introduced by E. R. Clark.)
24. *Sections showing the comparative vascularity of Purkinje fibers and heart muscle in the calf's heart.* P. N. JOHNSTONE, F. H. WAKEFIELD, and H. M. CURREY, University of Missouri. (Introduced by E. R. Clark.)
25. *Microscopic preparation of spleen and intestine of frog.* H. E. JORDAN and C. C. SPEIDEL, University of Virginia, Medical Department.
26. *Microscopic preparations of kidney, spleen, and limbs of normal and thyroid-fed frog larvae.* H. E. JORDAN and C. C. SPEIDEL, University of Virginia, Medical Department.
27. *Preparations showing the vascularity of the enamel organs of the white rat, the rabbit, the pig, man, and others.* H. M. KINGERY, School of Medicine, University of Colorado.
28. *Some recent developments in apparatus for testing acuity of hearing both for air- and bone-transmitted sound.* F. W. KRANZ, Wallace Clement Sabin Laboratory of Acoustics, Geneva, Ill. (Introduced by A. G. Pohlman.)
29. a) *Microscopic preparations showing synapses in the myenteric and submucous plexuses.* b) *Microscopic preparations showing the extrusion of aggregate of cells from the mucosa into the lumen of the stomach and intestine in Rana pipiens and Amblystoma tigrinum during metamorphosis.* ALBERT KUNTZ, St. Louis University School of Medicine.
30. *Nerve-terminations and plexuses in the mammalian lung.* Methylene-blue preparations. O. LARSELL, University of Oregon Medical School.
31. *The histological significance of the so-called germinal centers of lymphatic nodules.* JOHN STEVENS LATTA, University of Nebraska Medical School.
32. *Models of cells in elder-pith (Sambucus canadensis), $\times 500$ diam.* F. T. LEWIS, Harvard Medical School.
33. *Graphs and curves and tables illustrating the quantitative changes of the blood corpuscles of man in the first week after birth.* H. F. LIPPMAN, Anatomical Laboratory, University of Minnesota. (Introduced by R. E. Scammon.)
A summary of the complete blood findings on thirty-six infants in the first half of the neonatal periods, including the absolute and relative numbers of the red blood corpuscles, the various varieties of leucocytes and the blood platelets. The tabulations and graphs include the medians, averages, and ranges, and the standard (mean square) deviation and coefficient of variation of each value for each period.
34. *The parthenogenetic development of the egg in the ovary of the guinea-pig.* LEO LOEB, Washington University Medical School. (Introduced by L. H. Weed.)
35. *Model of human skull, enlarged, for teaching the head bones.* FREDERICK P. LORD, Dartmouth Medical School.
36. *Preparations of pancreas of guinea-pig to show changes in mitochondria due to inanition and refeeding.* WEN CHAO MA, University of Chicago. (Introduced by R. R. Bensley.)
37. *Injections of the kidneys.* D. B. MACCALLUM, University of Chicago. (Introduced by R. R. Bensley.)
38. *A new type of dissecting table (original design).* C. F. MCCLINTIC, Department of Anatomy, Detroit College of Medicine and Surgery. (Introduced by C. R. Bardeen.)

39. *Demonstration of fixed and living tissue cultures.* ALEXANDER A. MAXIMOW, University of Chicago.
40. *Changes in the histological structure of the stomach and the intestine of the albino rat, underfed for various periods.* SHIRLEY P. MILLER, Department of Anatomy, University of Minnesota.
41. a) *Models showing atria in the lung of the cat, the dog, and of man.* b) *Models of individual atria, of atria with air sacs attached, and sections showing atria.* WILLIAM SNOW MILLER, University of Wisconsin.
42. *Models, charts, and sections of the brain of Coenolestes obscurus.* JEANNETTE BROWN OBENCHAIN, University of Chicago. (Introduced by C. Judson Herrick.)
43. a) *Local development of pseudo-eosinophils in normal adult rabbit.* b) *Erythropoiesis in omentum of normal young rabbit.* EMILY PAYNE, Department of Animal Biology, University of Minnesota. (Introduced by Hal Downey.)
44. *Marchi preparations of the brain stem of the cat, showing the course of the tectospinal fasciculus.* A. T. RASMUSSEN, University of Minnesota.
45. *Studies of living blood-cells.* FLORENCE R. SABIN, Johns Hopkins Medical School.
46. *Curves, graphs, and formulae for expressing the relation between age and body-length in the fetal period of man.* RICHARD E. SCAMMON and L. A. CALKINS, Department of Anatomy, University of Minnesota.
47. *Field graphs, curves, and tables illustrating the postnatal growth of the entire brain and its parts and of the spinal cord.* RICHARD E. SCAMMON and HALBERT L. DUNN, Department of Anatomy, University of Minnesota.
48. *Preparation to illustrate the structure and distribution of Langergans islet elements in the pancreas of the frog.* L. C. SHEFFIELD, University of Chicago. (Introduced by R. R. Bensley.)
49. *Graphs and reconstructions (made by the orthoscopic method) of the topography of the female pelvic organs from the third fetal month to birth.* S. B. SOLHAUG, Department of Anatomy and Department of Obstetrics and Gynecology, University of Minnesota. (Introduced by R. E. Scammon.)
50. *The gastric glands of the alligator.* F. H. STALEY, Washington University. (Introduced by R. J. Terry.)
51. *Cleared rat fetuses, showing ear labyrinth.* R. M. STRONG, Department of Anatomy, Loyola University School of Medicine.
52. *The relations at puberty of the epiphyses to the joint cavities.* W. E. SULLIVAN, F. D. GEIST, and G. G. MUELLER, University of Wisconsin.
53. a) *Sections and drawings to show developing fiber tracts in nervous system of embryos of toad-fish (Opsanus tau).* b) *Sections and drawings of fiber tracts in spinal cord of early toad-fish larva.* c) *Model of brain of 30-somite toad-fish embryo.* HENRY C. TRACY, University of Kansas.
54. *The structural unit of the kidney.* HERBERT F. TRAUT, Johns Hopkins Medical School. (Introduced by Florence R. Sabin.)
55. *Dissections and drawings illustrating the internal anatomy of the forebrain of the opossum.* EDWARD L. TURNER, University of Chicago. (Introduced by C. Judson Herrick.)
56. *Preparations of pancreas of guinea-pig to show changes in mitochondria produced by vitamin deficiency.* HOWARD WAKEFIELD, University of Chicago. (Introduced by R. R. Bensley.)

57. a) *The demonstration of mitochondria in tissue smears.* b) *Demonstration of mitochondria grown in artificial culture media.* IVAN E. WALLIN, University of Colorado School of Medicine.
58. *Injected specimens showing the development of blood vessels in the medulla and midbrain of chick embryos.* ROY G. WILLIAMS, University of Missouri. (Introduced by E. R. Clark.)

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AN HISTOGENETIC STUDY OF THE RESPIRATORY EPITHELIUM

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THREE TEXT FIGURES AND THREE PLATES

The beginnings of physiological activity in any organ offer a most interesting field for study. Frequently in this rather neglected period are encountered features, the analyses of which contribute considerably to our understanding of the later structure of the organ. Recent studies of Policard and others of the Lyons school offer ample evidence for this assertion. Of all the bodily structures the lung undergoes perhaps the most obvious evolution at the time of, or shortly preceding, the birth period.

The degree of this evolution is universally admitted, but the time consumed in its accomplishment and the mechanism involved in its production are problems of some dispute. Since the matter has received but scant attention during the last three decades, available literature is rarely quoted, nor is it necessary at the present time to do more than briefly scan the field for purposes of orientation. From approximately 1870 on, students have been ranged in two schools. Küttner ('76) asserted that the respiratory epithelium remained cuboidal until flattened and distended by the first respiratory movements. His method consisted in first silvering the epithelium by instilling silver nitrate, and subsequently injecting gelatin. Upon examination it was noted that the portion of the lung uninjected with gelatin showed a cuboidal epithelium, whereas in the injected region the epithelium was flattened. Küttner's view is adopted by Jalan de la Croix ('83), the latter terminating his paper with the expression of a devout hope that he has once for all settled the question.

Colberg ('67), Laguesse ('86), and Stieda ('78) have maintained that the process of thinning out of the respiratory epithelium is a gradual one, and their view is perhaps the one generally accepted. Examination of the late fetal and early postnatal lung in a series of embryos of the albino rat convinced the writer, however, that certain features hitherto undescribed demanded attention.

The study was carried on during two summers while I was a guest of the Department of Histology at Ithaca, and the courtesy and counsel of Doctor Kingsbury are gratefully acknowledged. Material was obtained from The Wistar Institute rat colony. Litters were taken at 18, 18½, 19, 19½, 19¾, 20, 20½, 20¾, and 20 days 23 hours. Lungs of young rats aged one, two, four, five, six, ten, and twenty-one days, as well as adult lungs, were examined. Zenker's, Helly's, alcohol, Bell's fluid, Carnoy's, Benda's, Flemming's, Regaud's, and Altmann's fixations were employed. Frozen sections were made from fresh or formalin-fixed tissue for Herxheimer's stain. Iron hematoxylin was used as a routine stain. Altmann's anilin water acid fuchsin followed by either picric acid or methyl green proved very useful in some instances. My practice after some experimentation is to stain in 20 per cent anilin water acid fuchsin for five minutes steaming continuously, blotting, and differentiating with a mixture of saturated alcoholic and saturated aqueous picric acid equal parts; or in case methyl green is used, to stain until very deep green with a 1 per cent solution, washing out later the excess of green by immersing successively in water and 95 per cent alcohol. One has to determine in what condition the lungs shall be fixed. If one attempts to inject fixer to improve fixation, the pressure may be sufficient in itself to distend the lung. In many instances, however, I have followed this procedure, using embryos fixed by immersion after the thorax was opened and embryos fixed and sectioned as a whole as a check on the method. These latter are only available up to the twentieth day.

It is obvious that one has two factors to consider: first, the parenchyma, and, secondly, the stroma, i.e., the tissue compressing and that compressed. It is difficult to conceive the

lung parenchyma as one finds it, for example, in a seventeen-day rat embryo—a columnar epithelium with oval nuclei and densely granular cytoplasm, betraying no trace of special modification—passing suddenly with the first postnatal respirations to a flattened non-nucleated plate form without some very special adaptation becoming evident. In speaking of the first inspiratory movement, one is never on safe ground. It cannot be proved for a certainty that the fetus in utero does not engage in spontaneous gasping movements; indeed, if the musculature of a term rat uterus be cut through, exposing the contents still within the membranes, in many instances the thoracic walls may be seen to expand periodically. Embryos of nineteen days and over almost invariably gasp a few times when removed from the uterus. The extreme irritability of the respiratory mechanism at nineteen and one-half days may be appreciated from the fact that one embryo continued to have periodic expansions of the thoracic walls at some twenty-second intervals for seven minutes after the entire body below the diaphragm and the lungs themselves had been removed. Wislocki ('20) has shown that the lungs are colored blue after the injection of iron ammonium citrate and potassium ferrocyanide into the amniotic cavity, fixing with acid formalin. The writer is able to confirm this observation, and would therefore assume with Wislocki that communication exists between the amniotic cavity and the lumen of the bronchial tree. Inasmuch as in one case this diffusion of injected solution into the lung occurred within fifteen minutes and was complete throughout, one may probably assume with safety that it was aided by inspiratory movement on the part of the fetus. The matter of the relation of the amniotic fluid to the lung will be considered later in detail. In view of the fact of this communication, it becomes pertinent to inquire whether the force of uterine contraction transmitted to the fluid surrounding the embryo during labor may not assist in the preparatory transformation of the lung. These actual mechanical factors are difficult to prove or disprove. I know of no way by which we may make sure that a fetus does not gasp in utero, and in fact it probably always does. In view of this fact, the actual significance of the

mechanical pressure from inspiration upon the form of the lung epithelium will probably long continue to be a mooted point. One or two accessory factors in the expansion process may, however, be cleared up in the present study.

In seventeen-day rat embryos the smaller ramifications of the bronchial tree are lined by quite tall columnar cells. Nuclei are situated in the basal portion of the cell—that farthest from the lumen; they are oval in shape, are deeply staining, and contain from one to three nucleoli. The large number of mitotic figures observable is most striking; one cannot examine the cross-section of one of the smaller bronchial branches without encountering from one to six karyokinetic figures. There is no evidence of degenerative process at work and the spaces between the epithelial tubules is filled by fairly dense mesenchymal stroma. In fact, more of the lung volume is stroma than is epithelium.

During the eighteenth day, however, a marked alteration becomes apparent. This change concerns both the epithelium and the stroma. The latter becomes a much less conspicuous portion of the lung picture, having undergone a relative diminution as regards the epithelium, and whereas there is as yet no actual destructive process in progress, nevertheless the stroma is filled with osmic acid stainable fat-droplets. These droplets are likewise stainable with sudan III and Herxheimer's stain. The epithelium has undergone a still more noteworthy change. No matter what fixing agent is employed, the cytoplasm of the epithelial cell no longer appears granular in character. On the contrary, it is clear, transparent, homogeneous, hydropic-looking. In lungs fixed by Altmann's or Benda's methods the cytoplasm may appear to be filled with large globules of transparent structure. Mitochondrial structures are present. These are in the form of short rods and are distributed evenly throughout the cytoplasm. We shall return to the discussion of these clear cells below. The nucleus no longer occupies its characteristic basal position in the cell. In some cells the nuclei are swollen until they seem to fill the entire cell; again they are eccentric, lying in contact with the cell wall or in either corner of a cuboidally shaped cell; more rarely they appear compressed into a crescentic mass at

its base. Rarely, too, a non-nucleated cell is to be seen, though as yet apparently no general nuclear loss is in progress. In isolated regions the epithelium appears flattened, somewhat as if the tall columnar cell were turned over upon its side. Mitoses are less common and the impression afforded is that there is some decline in the growth rate.

By the nineteenth day each alteration in the earlier character of the lung becomes accentuated. The fat-content of the connective tissue is much increased. In Altmann preparations the mitochondria of the epithelial cell are seen gathered in certain regions, i.e., frequently at the periphery and at the base beneath the nucleus, the balance of the cell having the same peculiar clear appearance. The following explanation suggests itself. The hydropic character of the epithelial cell may mean that the amniotic fluid, known to exist within the lumen of the bronchial tree, is being cleared out through the activity of the lining epithelium in preparation for aerial respiration. It seems, therefore, desirable to see if the epithelial lining of the respiratory passages will absorb. Wislocki (loc. cit.) has shown that substances injected into the amniotic cavity reach the lung; whether they answer our requirement of being taken up into the epithelium is a different matter. The method used by me consisted in anaesthetizing the pregnant adult by paraldehyde via the stomach. After from 2 to 4 cc. they will as a rule remain quietly anaesthetized for an hour. The amniotic cavity was then injected through the uterus with a mixture of equal parts iron ammonium citrate and potassium ferrocyanide, 2 per cent, after an equal amount of amniotic fluid had been withdrawn. The uterus was then kept warm for one hour, after which time the embryos were removed, the lungs dissected out and fixed in acid alcohol. Grossly, the lung will be found to be colored bright blue. On microscopic examination the respiratory passages are seen filled with the blue granules. The latter tend to precipitate along the walls and the peripheral cytoplasm of the epithelial cell is often stained a diffuse blue. From this diffuse staining no safe deduction can be made. At eighteen days nineteen hours, few granules were found in the lumina; none were found

within epithelial cells; a few were encountered within the connective-tissue spaces and in vessels. It is assumed that they entered the circulation from the digestive tract of the embryo, though no attempt was made to verify the supposition. At nineteen and one-half days, however, large numbers of granules occupied the lumina, and in more than a single instance they were seen within epithelial cells. They were likewise found within a phagocytic cell just under the epithelium. This of course does not mean that they passed the epithelium to be phagocytized, for the cell may well have lain out upon the surface of the lumen. They were not found within the connective tissue. In six embryos immediately before birth, injections into the amniotic cavity all failed to reach the lung.

Taken all in all, less evidence of absorption was found than was anticipated; it is quite possible, however, that our hydropic-appearing cells owe their appearance to a slow, prolonged absorptive process, continued over a considerable period. The fact remains that some absorption has been shown to have occurred. The quantity of this absorption of wholly foreign substances is of course no reliable index whatever as to the rate of absorption of physiological fluids. The clear cells do not give the staining reactions of mucin. In many instances they may be shown to be rich in glycogen. Both the iodine method and Best's method have been used and their results checked by digestion with saliva. The glycogen is not evenly distributed in the cell as a rule. Usually it is found as a huge eccentric globule. A large clear globule may persist in the cell completely surrounded by deep red-staining material or the clear globule itself may take on a pinkish cast. In the epithelial cells of the larger bronchi the glycogen is precipitated in granular form. Glycogen is present in the lung in large quantity up to the twentieth day. From this time on the histological evidence shows that it diminishes and in young animals aged two and five days, respectively, it proved almost entirely absent.

From the twentieth day on in addition to the clear, homogeneous appearance of the epithelial cell, certain other features attract one's attention. The mitochondrial filaments show a

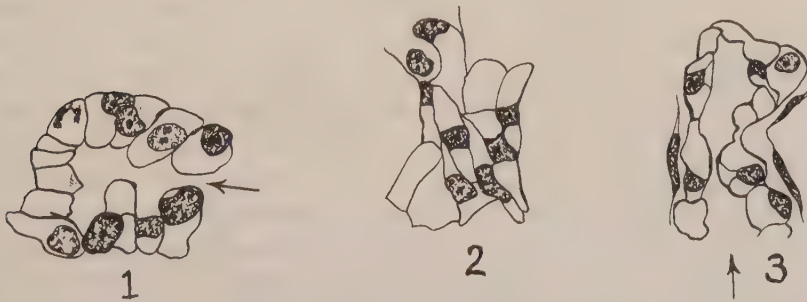
distinct tendency to group themselves into clumps and knots or to become swollen and to assume a round or dumb-bell shape. Certain of these larger fuchsinophile droplets reduce osmic acid to some extent and stain brownish red to black (figs. 31 to 37). How close the relationship is between the fats and mitochondria is well known, and its discussion would be out of place in this paper. It would appear merely that the mitochondria were in process of such alteration that their fatty constituent was free to reduce the osmic acid. Certain of the globules show clear centers surrounded by either black or fuchsinophile rings where the fixer has penetrated poorly. Some of them are colorable by Herxheimer's stain, doubtless those reducing the osmium tetroxide; indeed it would seem that the quantity of osmic acid stainable fat just about parallels the scharlach R stained droplets. That these apparent mitochondrial changes are not the result of poor fixation may be seen from the fact that the mitochondria in included tissues, such as the heart, larger bronchi, etc., are of the filamentous form. I have thought of these cytoplasmic changes as one aspect of the cell's transformation from a nucleated structure with a certain active metabolism into a more or less passive air filter. The alterations in these cytoplasmic constituents may likewise be in some way related to the fluid imbibition; the hydropic character of the cell may well influence its metabolism in an unfavorable direction. The fact that the active growth process seems to slow down markedly with the beginning of the clearing up of the cytoplasm, only to reappear again during the first day after birth, affords some circumstantial evidence in this direction.

At twenty days the flattening of the epithelium is very apparent. Figures 1, 2, and 3 are from camera-lucida drawings of the epithelium in a twenty-day embryo sectioned as a whole, where the lung could neither have collapsed to slight extent, as after opening the thorax, nor be distended as after the instillation of fixer into the trachea. Figure 1 shows an epithelial tube, lined by tall columnar cells, irregular in outline and tending somewhat to overlap one another, the nuclei assuming all possible positions. At the base of this somewhat horseshoe-

shaped figure is a row of cells without nuclei or else a row of cell processes cut distantly from the nucleus. Figure 3 represents another region from the same lung, where the epithelium is quite flattened, always retaining its clear appearance, however, and figure 2 illustrates a surface view of the same type of epithelium, showing how the flattened epithelial cells interlock with their plate-like processes. Some of these cells may be non-nucleated. In dealing with these long processes it is manifestly difficult to make certain whether one is observing a non-nucleated cell or whether it is a cell process cut some distance from its nucleus. One must be cautious in describing non-nucleated cells; in the study of their formation, I have been careful to choose those cells in which unmistakable nuclear fragments were present. Thick sections then become an advantage rather than otherwise.

By this time the stroma of the lung is loaded with osmic acid reducing fat-droplets of all shapes and sizes, and certain of the mesenchymal cells seem on the road to destruction. Before disappearance they may form confluent masses (fig. 4), in which masses the fatty element is most prominent. Some attention has been given to the origin of this fat, it being necessary to our interpretation to determine whether it arises from deposition in the connective tissue or whether it comes as an actual metamorphosis of the cytoplasm of the stromal cell. Figures 26 to 30 and 39, taken from a particularly favorable region in the lung of a five-day-old rat, will serve to illustrate. As in the case of the epithelium, there seems to be an intimate relation between fuchsinophile granules (mitochondria) and granules blackening with osmic acid. The amounts of the two in the cells are reciprocal; color transitions are evident. Figure 26 shows a cell containing fuchsinophile filaments, globules surrounded by a fuchsinophile ring or a ring staining reddish black to black where the osmic acid has colored a globule in which penetration of the fixer has been imperfect. Figure 27 represents a cell where penetration has been more perfect. In figure 28 fuchsinophile granules or filaments are absent and osmic acid reducing fat-droplets are present in large numbers. As in the case of the epithelium, the question arises as to whether or not these inter-

mediate forms between the fuchsinophile filament and the black droplet are the products of poor fixation. The ring forms of course indicate poor penetration; our question merely is, did the ring preexist as a fat-droplet before fixation? All evidence indicates that it did. Figure 30 illustrates the typical mitochondrial picture in a cell chosen from the connective-tissue sheath of a vessel which penetrated the mass from which cells taken for the previous illustrations lay. This seems to afford ample evidence that one is not dealing with poor fixation in the sense that substances which should have appeared as filaments actually appear as globules, for in this last figure the mitochondria are



Figs. 1, 2, and 3 Respiratory epithelium from lung of twenty-day rat embryo, cut and mounted in complete series. Zenker's fluid. An arrow designates the lumen. $\times 750$.

seen to be distinctly filaments. Evidence would indicate, therefore, that the fat stainable in the lung stroma arises in situ as a true fatty degeneration of the cell, an anticipatory reaction in the teleological sense, for the expansion of the epithelial portion soon to occur. The appearance of fat in the connective tissue is apparently unrelated to its forthcoming in the epithelium, i.e., there is no evidence that connective-tissue cells phagocytize fat-droplets formed in the epithelium, for the fat appears in the connective tissue before it is found in the epithelium. Abundant fat is found in the lung stroma up to ten days after birth, and even in the adult it is not entirely absent, although here its distribution is practically restricted to the tissue surrounding

the larger bronchi and to certain localized regions about which more may be said in a later connection.

One must of necessity face the question of the origin of the non-nucleated epithelial plates. Are they to be thought of as arising as processes of cells which later become segmented off from the nucleated portion of the cell? Do they originate through a process of nuclear loss? I believe the best evidence favors both of these modes of origin. Figures 5 to 12 and 22 show regions of the lung at different stages from embryos of the twentieth day up to young animals aged four days, where the nucleated epithelial cells possess greatly elongated flange-like processes, extending out from the nuclear region of the cell. These flanges may be many times the length of the nuclear region. Often the cytoplasm of these cell flanges seems crossed completely or partially by division lines (fig. 12). Certain of these division lines undoubtedly mark out processes from other cells and may not be considered as segmenting what was originally a single flange, but others leave a great deal of doubt. By simple deduction it would appear necessary to assume that these flanges did give rise by segmentation to the non-nucleated plate; they exist in the embryo and in the new-born; they may be found in the latter up to the fourth day at least, being traceable for long distances closely applied to the capillary wall and vanishing in an imperceptible thinness; they are not described in the adult; there is no reason to assume that they degenerate, and the logical supposition is that they give rise to the non-nucleated plate through a process of segmentation. That epithelial cells lose their nuclei and become compressed into epithelial plates is more easily ascertained. Figures 15 to 18 illustrate nuclear degeneration as found in various regions of the twenty-day fetal lung. Pycnoses are absent; the degeneration process seems one of fragmentation and lysis. Now and then a cell (fig. 14) with two nuclei (abortive division ?) is seen.

The process of nuclear loss does not terminate with the birth of the fetus. The lung of a two-day rat contains many degeneration figures, and they may be found in the four-day rat, though in markedly reduced numbers. Further than this they have not

been followed with adequate techniques. Figure 19 illustrates a clear flattened epithelial cell with a practically negative cytoplasm and a faintly staining mass of what seems to be nuclear material near its center. The same cell contains several large fat-droplets; evidently the process of fatty degeneration of the epithelial cell likewise does not cease with birth. This drawing is from the four-day lung. Figure 39 illustrates one of the large fat-laden epithelial cells from the adult lung. In it one finds fuchsinophile granules, pale globules staining gray with osmic acid and with a darker ring, and irregular deep black carbon particles (anthracosis).

To summarize briefly the foregoing: the lung epithelium becomes hydropic in the later stages of fetal life; with this hydropic transformation and possibly due to it, the cytoplasm of the epithelial cell undergoes a sort of fatty metamorphosis, the best evidence being that the fat arises *in situ* from fuchsinophile filaments at least in part; the cytoplasm becomes microscopically practically structureless; the connective tissue through a similar process of fatty degeneration in part disappears, after in some cases forming confluent fatty masses; thereby space is made for the expansion of the alveoli. The epithelial cell is either flattened by pressure brought about by gasping movements in utero or with the first postfetal inspirations, directly from a cuboidal form in some regions, and one should realize, no doubt, that the cuboidal epithelial cell possesses a cytoplasm rendered considerably more plastic through fluid imbibition—less firm and rigid and more capable of deformation. Besides this mechanical factor in the expansion one has the formation of the plate-like flanges which are assumed, partially through observation and partially by deduction, to give rise by segmentation to non-nucleated respiratory plates. In the process of expansion these various cells must either be sufficiently plastic to completely line the alveolus or else for a time some portion of the alveolar wall¹ must re-

¹ The expression 'alveolar wall' is used throughout this paper in a loose sense to designate any region whose structure is such that there is reason to believe it will later develop epithelium of the strictly respiratory type. It has been found unnecessary in the present investigation to attempt exact delimitation of anatomical regions.

main naked of epithelium. These points can scarcely be settled beyond doubt. Evidence, however, indicates that abundant loopholes may exist in the wall and that the capillary endothelium may be in direct contact with the air space. The subsequent relining of the alveolus then continues just as after a destructive pneumonic process—by the throwing out of flange-like processes. Objection may be raised that if this were true, the basis for an interstitial emphysema would be present—that air would be passing into the stromal spaces. Answer may be made that there never has been any evidence that air does not pass into the stroma normally. Else one would have to assume that only the portion of the epithelial plate in direct contact with a capillary wall is capable of acting as an oxygen transmitter. A glance at figures 20 and 21 will illustrate another method of lining the expanding alveolus; in these figures it is seen that the flange process of each cell overlaps the body of the next succeeding cell. Thereby a mechanism for the sliding by of the epithelial cell, as the alveolus expands, is provided. This is a very common finding.

Apparently not all of the cells which undergo fatty transformation in the modification of the alveolar walls actually persist as respiratory plates. Viewing their histological picture purely apart from the adaptative side, they really have much in common with a degenerated cell. They undergo hydropic change, fatty degeneration of their cytoplasm, and lastly they lose their nuclei. It is not remarkable, then, that one does encounter evidences of sloughing off of epithelial cells into the alveolus. This slough is seen to comprise both individual cells and small cell groups, and the cast-off cells come to lie within the lumen, in which they degenerate, giving rise to ill-defined masses containing much fat. This sloughing process is met with in slight degree before birth. It is evident in the lungs of two- and four-day rats and in the adult. Whether or not in the adult it is related to slight lung pathology I cannot say. In favor of its being in part pathological we have the fact that the sloughing at times is seen in localized patches, and in these patches the connective tissue may likewise be quite fatty. On the other hand, it is always present.

It occurs in the young as well as in the old, in the lungs of Wistar rats as well as of those raised under far less aseptic conditions.

Granel ('19 a and b) recently described the 'cellules à graisse' in the lung. Fauré-Frémiet ('20) described the cuboidal cells of the lung epithelium as containing large numbers of globules giving the solubilities of fat; microchemical tests led him to believe the globules were cholesterol. He stated that they could not be preserved by any paraffin technique. A glance at figure 24 is sufficient to show that this last statement is an error; nor would I agree with Fauré-Frémiet in saying that they reduce osmic acid feebly. The same figure shows a strong reduction of the osmic acid. This, however, may be said, I have found it impossible to secure uniform staining of the droplets; many appear unstained and are seen as bright refractile dots. Bell's method has given the best results, even better than scharlach R. The droplets are perfectly evident in the unstained frozen section with no fixation. They show prettily with the dark-field microscope. Certain it is that these fat-containing cells of the epithelium are the source of the desquamated cells above mentioned. These desquamated cells in the adult are nucleated cells. The writer would agree with Granel in his statement that similar cells may be encountered in the epithelium of the larger air passages (fig. 25). The significance of this fat in the pulmonary epithelium is quite problematic, though its quantity would suggest that its relation to the chemistry of the epithelial cell is more than a passive one.

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PLATE 1

EXPLANATION OF FIGURES

In all cases an arrow is used to designate the lumen.

4 Degeneration in the lung stroma; confluent fatty mass in lung connective tissue of one-day-old rat. Flemming's fluid. $\times 850$.

5 and 6 Flange-like processes of epithelial cells, blood capillaries, blood cells, and stroma cells. Rat embryo aged twenty days twenty-three hours. Flemming's fluid. $\times 950$.

7 and 8 Epithelial flanges, capillaries, and stromal cells from lung of young rat, aged four days. Flemming's fluid. $\times 670$.

9 to 12 Epithelial flanges in two-day-old rat lung. *Fl.*, flange; *Lum.*, lumen; *Cap.*, capillary; *Fl. Br.*, line of breakage in flange; *Epi. Nuc.*, epithelial nucleus; *End.Nuc.*, endothelial (capillary) nucleus; *c*, cell with distinct cuticular border surrounding clear globule. Flemming's fluid. $\times 670$.

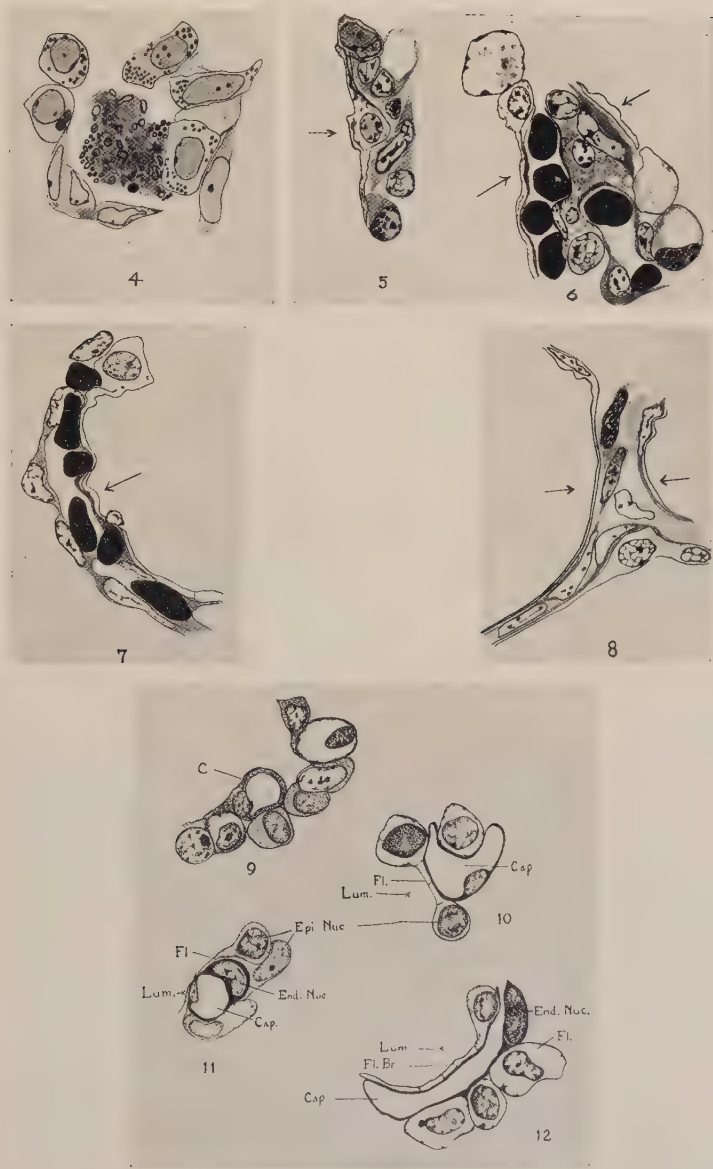


PLATE 2

EXPLANATION OF FIGURES

In all cases an arrow is used to designate the lumen.

13 Normal epithelial cell from lung of twenty-day rat embryo. Flemming's fluid. $\times 850$.

14 Binucleated cell from lung of same embryo. $\times 850$.

15 to 18 Nuclear degeneration figures from same lung epithelium as figures 13 and 14. Flemming's fluid. $\times 850$.

19 Flattened epithelial cell from lung of rat aged two days; *n*, nuclear remains; *f*, fat-globules; the cytoplasm is otherwise almost clear. Flemming's fluid. $\times 850$.

20 and 21 Overlapping flanges in epithelium of twenty-day fetal rat lung. Flemming's fluid. $\times 850$.

22 Non-nucleated cells (*c*) from the epithelium of the lung of new-born rat (during the first day). Flemming's fluid. $\times 850$.

23 Fat-containing cuboidal epithelial cell compared to adjacent degenerated cell found in lumen. Embryo aged twenty days and twenty three hours. Flemming's fluid. $\times 1700$.

24 Osmic acid reducing fat in the adult lung. Bell's fixation; paraffin imbedding; unstained. $\times 340$.

25 Fat-containing cells in wall of bronchiole; rat aged two days. Flemming's fluid. $\times 335$.



PLATE 3

EXPLANATION OF FIGURES

26 to 30 Cells from the stroma of five-day-old rat lung. Altmann's technique. $\times 1700$.

31 to 33 Epithelial cells showing typical mitochondrial picture at eighteen days nineteen hours, nineteen and one-half days, and twenty days eight hours, respectively. Altmann's fixation. $\times 1700$.

34 to 37. Process of fat formation in epithelial cells of the lung in fetus of twenty days eighteen hours. Altmann's fixation in 35, 36, 37; Flemming's fluid in 34. $\times 1700$.

38 Mitochondria in the cuboidal type of cell of the respiratory epithelium in ten-day-old rat. Altmann's fixation. $\times 1700$.

39 Epithelial cell about to desquamate; adult lung. Altmann's fixation. $\times 1700$.



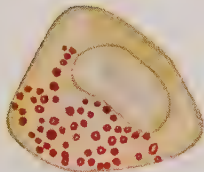
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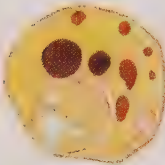
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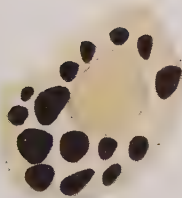
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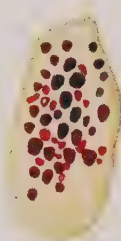
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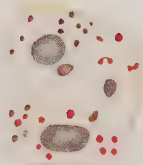
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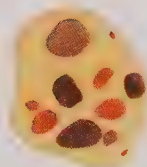
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A CONTRIBUTION TO THE ANATOMY AND THE DEVELOPMENT OF THE ARTERIAL AND VENOUS SYSTEMS IN TURTLES

PAULINE KIMBALL

THIRTEEN FIGURES

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1. INTRODUCTION

Most of the papers on the embryology of the blood vascular and lymphatic systems have dealt with the more prominent arteries, veins, or lymphatics, such as the postcava, the renal portals, the postcardinals, the thoracic duct, etc. Due to the large amount of degeneration of the embryonic arteries and veins in the central region, the difficulties involved in fixation and reconstruction of these vessels, and the shrinkage factor, it was deemed advisable to study some of the more superficial vessels, such as those of the lateral body or vertebral regions. It was the original plan of the writer to study the origin and the development of the lymphatics of these regions, but before going very far with the lymphatic problem, it was found necessary first to study the anatomy and development of the blood vessels in these particular regions. Obviously, any work upon lymphatics which is not founded upon a sound knowledge of the vascular anatomy of the particular region studied is likely to be faulty.

It is the purpose of this paper, first, to make a detailed study of the adult anatomy of the blood vessels and, second, to work out their embryonic development. The blood vessels with which

this paper will deal are: the marginocostal, the vertebral, the intercostal arteries and veins, and the renal portal vein. The anatomy of these vessels and their location will be discussed under section 4.

2. METHODS OF INVESTIGATION

The material for this work was collected at the Iowa Lakeside Laboratory, located on Lake Okoboji, in northwestern Iowa. The summer of 1921 some forty turtle nests were found not far from the laboratory grounds. The following summer, 1922, over seventy-five nests were found in the same place. With such an abundance of material so close at hand, it was easy to collect as much material as was desired and at almost any interval. Embryos have now been collected from two days' incubation until hatching.

The embryos collected were of the turtles *Chrysemys marginata belli* (Gray) and *Chelydra serpentina* (Linnaeus), the snapping turtle. It was found that the rate of incubation depends to a great extent upon the location of the nest and upon the temperature. The summer of 1922 was unusually cool in comparison with the preceding summer. As a result, development was slowed down considerably during 1922. Two trees shaded one end of the plot of ground in which some of the nests were located in such a way that the sun was only able to warm the ground for a few hours each day. Obviously, there would be a difference in the rate of development between the nests located in the shade and the nests located in an open spot. Due to these conditions, measurement of the embryos was decided upon as being a more reliable method for denoting stages than by keeping track of the age. Another factor which led to the adoption of the measurement method was the small number of eggs found in the nest of the turtle *Chrysemys marginata belli*. The number varies from five to fifteen. The usual number is seven or eight. To collect a well-injected series for any length of time from one nest would be obviously impossible. All embryos were carefully measured and outline drawings were made of them with the aid of the camera lucida.

Ordinary black water-proof India ink was used as an injection mass. The instruments used for injecting were very simple, a small piece of rubber tubing attached to a very fine glass canula was found to be satisfactory. A small low-power dissecting microscope was used to aid in placing the canula in the proper spot.

Injection in the older embryos was made, as a rule, in the neck region through the carotid artery. The umbilical veins also proved quite satisfactory, especially so after the skin had begun to toughen and the shell to harden. The younger embryos were almost all injected through the vitelline veins.

The fixing agent used was chromo-aceto formaldehyde.¹ The following is the formula for the fixing agent:

Chromic acid, 1 per cent,	64 parts
Acetic acid (glacial),	4 parts
Formaldehyde, pure (40 per cent),	32 parts

The embryos were stained according to the usual procedure. An acid haematoxylin was used instead of a plain aqueous solution. This was made by adding 10 per cent nitric acid drop by drop to the Delafield's haematoxylin until the proper acidity was obtained. This can only be determined by stirring constantly and holding the jar up to the light. When the solution begins to clear, sufficient nitric acid has been added. A slightly acid haematoxylin solution seemed to penetrate the tissues better than ordinary Delafield's haematoxylin.

A special counterstain was used. It was made up as follows:

Erythrosin, saturated alcoholic solution,	50 cc.
Aurantia, saturated alcoholic solution,	10 cc.
Orange G, saturated alcoholic solution,	40 cc.

Erythrosin alone was not found to be satisfactory for embryonic tissues. It was found that alcohol washed out the erythrosin almost immediately, no matter how long the slides were allowed to remain in the stain.

¹ Lee. The Microtome's Vade-mecum. 4th ed., p. 55.

Norris. Proceedings of the Iowa Academy of Science, vol. 8, p. 78 (1900).

Stromsten, F. A. Carnegie Pub., no. 132, p. 80.

3. HISTORICAL

The literature concerning the development of the superficial vessels of the body wall of vertebrates consists of observations given, in a great part, as isolated or unexplained steps. No work has been done upon the vessels of the lateral body region of the turtle. The embryology of certain vessels which might correspond to the ones in this region has been studied and reported upon in other vertebrates by Kampmeier ('20)² (Anura) and Smith ('09) (pig).³ Other writers have been more interested in the development of the lymphatics, so have only mentioned the blood vessels incidentally. Considerable confusion in the names of the adult vessels in the curve of the carapace has been found. Hyman ('22)⁴ calls the vessels in the curve of the carapace the marginocostals. Pratt ('05)⁵ calls them the internal mammaries. Both writers agree that these vessels join the epigastrics posteriorly. Smith (pig) calls the vessel draining the lateral body wall the thoraco-epigastric. Lewis⁶ (rabbit) calls it the external mammary. Later he adopts Smith's term, thoraco-epigastric. Lewis makes the following interesting statement:

The thoraco-epigastric vein which is found in the lateral body wall is represented in fishes and in all the higher classes of vertebrates. Unfortunately it has been given a great variety of names. In the rabbit it is called the external mammary vein (Krause,¹ Lewis²) and this name has been applied to it in man (Poirier³). In man it has been called, in part at least, the long, lateral, or inferior thoracic vein, but the term thoraco-epigastric is preferable to any of these.

It would in any case be a question as to whether the thoraco-epigastric of Smith (pig) and Lewis (rabbit), the marginocostal of Hyman (turtle) or the internal mammary of Pratt (turtle) and the lateral vein of Kampmeier (Anura) are identical in history and function with the lateral vein of the fish.

Smith's paper contains perhaps the most detailed account of the vessels in this region. Her studies were made upon the pig.

² Kampmeier, O. F. *Anat. Rec.*, vol. 19, pp. 83-97.

³ Smith, H. W. *Am. Jour. Anat.*, vol. 9, no. 3, pp. 439-469.

⁴ Hyman, Libbie H. *A Laboratory Manual for Comparative Anatomy*.

⁵ Pratt, H. S. *Vertebrate Zoology*.

⁶ Lewis, F. T. *Am. Jour. Anat.*, vol. 9, no. 1, p. 33.

Unfortunately, she has paid more attention to the changes which occur in the circulation of the blood in the lateral body wall than to the details of anatomy of the vessels. Her conclusions are as follows: There are four stages in the development of the circulation of the body wall. The first stage is the stage in which the body wall is drained chiefly by the postcardinal vein. The second stage shows the body wall drained chiefly by the umbilical vein. The intersegmental arteries feed the capillaries which empty into the umbilical vein.

The third stage is the formation and growth of the thoraco-epigastric and superficial epigastric on the outer side of the muscle layer and somewhat later that of the internal mammary veins and artery on the mesial side. The thoraco-epigastric grows with the superficial epigastric until they drain the whole superficial body wall dorsalwards as far as the spinal cord. Meanwhile the internal mammary vessels have grown. The intercostal veins and arteries have also been spun out of the plexuses of the segmental vessels, as the muscles and ribs invade the membrana reuniens, and they anastomose with the anterior intercostals from the internal mammary vessels. The internal mammary vein increases in size. Its connections with the thoraco-epigastric increase also and gradually the end of the thoraco-epigastric is switched off and we reach the fourth and final stage, which the superficial body wall drains largely into the internal mammary vein. After this condition is reached, further change is not so much in kind as in degree.

Lewis ('05)⁷, in his paper upon the development of the lymphatic system in rabbits, incidentally mentions the veins of the lateral body region. He calls these veins the external mammaries. Later ('09) he changes the name to thoraco-epigastric. In speaking of these vessels, he says: "Where the external mammary vein joins the brachial there is a large sac, and the question arises whether or not the detached lymphatics following the mammary vein are independent formations, or are outgrowths from that sac." His use of the term brachial might be questioned. From his diagrams it looks as though the brachial and external mammary were branches of a joint vessel, the axillary. He makes another statement regarding the veins of the lateral body region: "The body wall is supplied by those (lymphatics) which

⁷ Lewis, F. T. *Am. Jour. Anat.*, vol. 5, no. 1.

follow the external mammary vein in its anastomosis with the superficial epigastric and by vessels accompanying the ilio-lumbar vein." Since no further mention is made of the superficial epigastric or any reference made to it in his figures, it is difficult to determine just how anastomosis with the superficial epigastric has occurred. Judging from his figures, the femoral vein seems to be the ultimate destination of blood which passes through this region. The embryos which he studied range in size from 9.5 mm. to 29 mm. From a study of his eight figures, it would seem that he might offer the hypothesis that the external mammary sprouts from the axillary in the armpit, gradually invades the body wall and ultimately joins posteriorly the femoral. This is interesting in that it offers one hypothesis for the formation of the marginocostal vessels of the turtle. We shall soon see that this hypothesis is incorrect.

Polinski ('10)⁸ made a study of the lymphatics of the calf. He speaks of the lymphatics of the lateral body wall, but the only mention which he makes of the blood vessels is in reference to the work of Lewis and Smith.

Stromsten ('05)⁹, studying a different species of turtle, reports that "The vertebral veins develop as a longitudinal fusion of the dorsal intersegmental branches of the postcardinals above the costal anlagen." He also reports that the caudal portion of the postcardinals are retained as the anterior and posterior advehent veins of the permanent kidneys. The latter are the renal portal veins which are discussed in this paper.

According to Kampmeier, the lateral vein in *Anura* develops as follows:

During development all of the intersegmental veins back of the anterior lymph hearts become interjoined by a longitudinal anastomosis which may be termed the lateral vein because it courses in the lateral-line region and is without doubt homologous with a similar vein in the tailed amphibians. Subsequently, the termini of all intersegmentals except the 9th and 11th in toad embryos become very much reduced or vanish, although variations happen, such as the persistence of the vessels in intervals other than those.

⁸ Polinski, Wladyslaw. Bull. Acad. d. Sci., Cracovie.

⁹ Stromsten, F. A. Am. Jour. Anat., vol. 4, no. 4.

A part of the lateral vein forms the dorsolumbar veins of the adult. The 7th intersegmental vein "becomes the transverse piece or mouth of the dorsolumbar vein." As to the 11th intersegmentals, "the proximal portion or juncture of the latter branch with the post cardinal (vena renalis advehens) becomes the mouth or terminal segment of the posterior vertebral vein and the caudal part of the lateral vein becomes its distal extension."

As to the formation of the renal portal vein, Kampmeier says:

The original condition of the postcardinal lying against the medial side of the primary excretory tube of the Wolffian duct, is soon altered by the formation of a second channel on its lateral side as the result of longitudinal anastomoses between the intersegmental veins near their points of confluence with the postcardinals.

These lateral channels are the renal portals of the adult. Kampmeier calls them the Venae renales advehentes (Jacobosen's veins):

4. ADULT ANATOMY

Before taking up the embryology of the marginocostals, the vertebrals, the intercostals, and the renal portals, it will first be necessary to discuss their adult anatomy. As an aid to the following description, refer to figure 1, which is a diagram of the arteries in question, and to figure 2, which is a diagram of the veins. The turtles *Chelydra serpentina* and *Chrysemys marginata belli* were both dissected. Hyman's Laboratory Manual ('20) was used as a guide in dissection.

The arteries

The innominate artery, upon leaving the right aorta, divides into four branches, the right and left subclavian (outer) and the right and left carotid (inner). Following one of the subclavians out into the armpit where it is known as the axillary, several small branches are given off. One of these is the vertebral and another is the marginocostal. The vertebral swings up and in front of rib I makes a sharp turn posteriorly running dorsal to the transverse processes from ribs I to IX. Here a branch of the epigastric artery (a direct branch of the dorsal aorta) swings

up dorsally to meet it. The intercostal connections between the aorta and the vertebral arteries, found in mammals and other vertebrates, are absent in the turtle. The degeneration of these vessels has probably been brought about, in part at least, by the development between the vertebrae and the aorta of the large retractor muscles which function in retracting the head and neck. The two aortae join ventral to and on a level with ribs III to form one continuous vessel. The bifurcated condition of the aorta anterior to this point would explain the degeneration of the first two pairs. The intercostal arteries lie between the ribs, are seven in number, and arise as branches of the vertebral artery. Their function is to supply the carapace with blood. In the curve of the carapace, they join the marginocostal artery. The marginocostal artery, mentioned before as a branch of the axillary, runs in the curve of the carapace and joins the epigastric posteriorly.

The veins

The vertebral vein occupies a position ventral and lateral to the vertebral artery. It joins the renal portal by a prominent branch between ribs V and VI. The first four intercostal veins arise as branches of the vertebral vein. Sometimes the fourth intercostal veins come off the renal portal just posterior to the point where the branch from the vertebral vein joins the renal portal. The last three intercostal veins come off the renal portal vein directly. The iliac, a posterior continuation of the renal portal, is formed by the juncture of the femoral and abdominal veins. Just anterior to this juncture, the iliac gives off the epigastric vein which runs to the edge of the carapace where it turns anteriorly and joins the marginocostal vein. The marginocostal vein lies ventral to the marginocostal artery in the curve of the carapace. Anteriorly it is continuous with the first intercostal vein. It has no axillary connection as does the marginocostal artery.

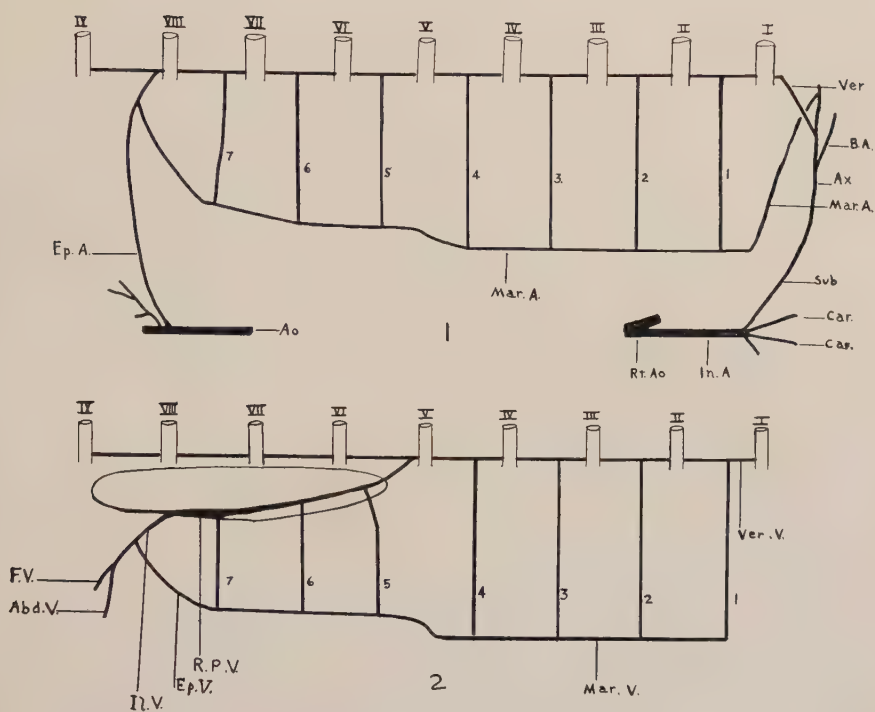


Fig. 1 The adult arteries.

ABBREVIATIONS

Ao., aorta
Ax., axillary
B.A., brachial artery
Car., carotid arteries
Ep.A., epigastric artery
In.A., innominate artery

Mar.A., marginocostal artery
Rt.Ao., right aorta
Sub., subclavian artery
Ver., vertebral artery
1 to 7, intercostal arteries
I to IX, ribs

Fig. 2 The adult veins.

ABBREVIATIONS

Abd.V., abdominal vein
Ep.V., epigastric vein
F.V., femoral vein
Il.V., iliac vein

Mar.V., marginocostal vein
R.P.V., renal portal vein
Ver.V., vertebral vein
1 to 7, intercostal veins
I to IX, ribs

5. EMBRYOLOGY

Embryologically, these vessels just described originate as follows: The vertebral arteries are formed by a longitudinal anastomosis of the branches sent dorsally into that region by the intersegmental arteries. The vertebral vein is formed in the same way by the longitudinal anastomosis of the intersegmental veins in the vertebral region.

The marginocostal arteries and veins develop by a longitudinal anastomosis in the curve of the carapace of the branches sent out into that region by the intersegmental (intercostals of the adult) vessels from the aorta and postcardinal veins, respectively.

The intersegmental arteries and veins anastomose freely as they do in the adult. It is out of these plexuses that the intercostal arteries and intercostal veins of the adult are formed.

The postcardinal veins degenerate as far back as the 5th ribs. The posterior portions remain throughout life. In the adult these are called the renal portal veins.

The youngest stage studied measured 9 mm. (crown-rump measurement) and is listed as serial no. 40. Figure 3 is a diagram of the arteries in question; figure 4 is a diagram of the veins. The embryo was cut into cross-sections of 35 μ thickness. Drawings of every section were made with the projection lantern and later studied more carefully with both the high and low power of the microscope. A reconstruction was made upon graph paper. Each square was made to represent one particular section and numbered according to slide, row, and number of the section in the row. The diagrams, represented by figures 3 and 4, were made from these reconstructions.

It will be noted from the diagram that the intersegmental vessels and their branches are more prominent in the anterior region. This is to be expected, since growth proceeds caudally. The branch which is sent out into the lateral body region from the axillary artery should be noted. This is the vessel which comprises the anterior portion of the marginocostal artery. There are eight pairs of intersegmental arteries. These arise from the dorsal aorta, rise to a position on the level and lateral to the notochord, where they fork. One branch passes dorsally

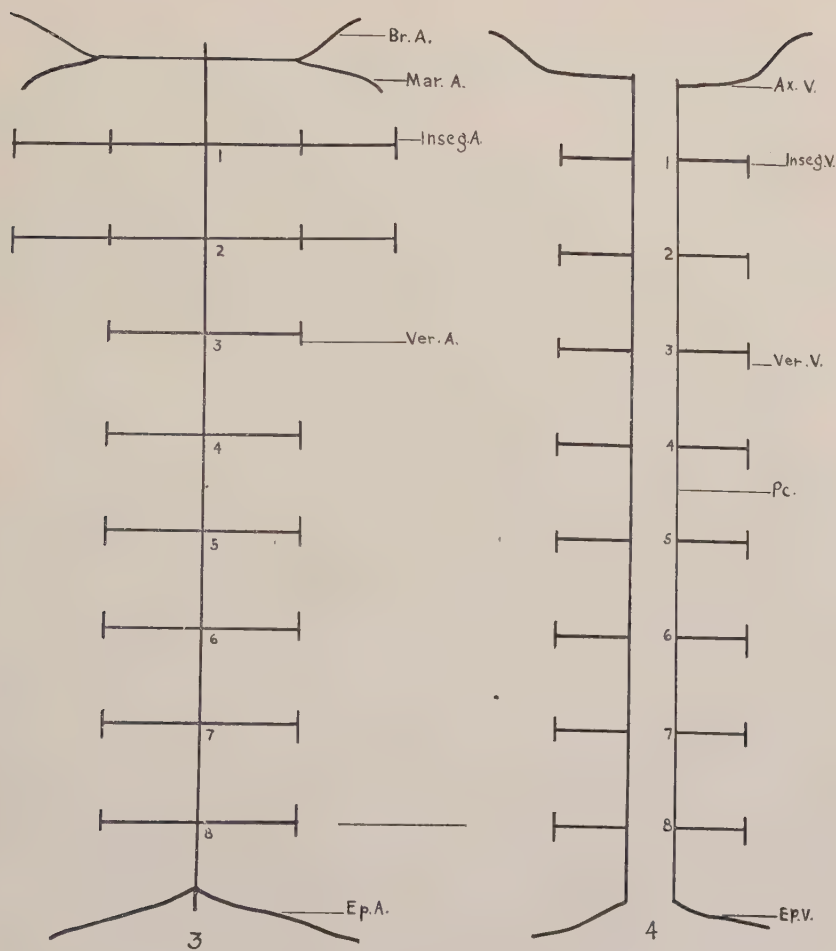


Fig. 3 The arteries. Serial no. 40.

ABBREVIATIONS

Br. A., brachial artery*Ep. A.*, epigastric artery*Inseg. A.*, intersegmental arteries*Mar. A.*, marginocostal artery (beginning of)*Ver. A.*, vertebral artery (beginning of)

Fig. 4 The veins. Serial no. 40.

ABBREVIATIONS

Ax. V., axillary vein*Ep. V.*, epigastric vein*Inseg. V.*, intersegmental veins*P. C.*, postcardinals*Ver. V.*, vertebral vein (beginning of)

into the region which is later occupied by the vertebral artery, and the other branch runs out into the lateral body region.

The postcardinal veins are continuous throughout, entering anteriorly the sinus venosus. They resemble the intersegmental arteries in that they fork at the same level as do the intersegmental arteries, sending one branch into the vertebral region and the other toward the lateral body region. It was not possible to trace these vessels very far into the lateral body region. The future vertebral vein develops at the fork. This is not true of the vertebral artery. The vertebral artery develops higher up. Figure 7 shows the relationship of the intersegmental vessels.

The second series studied was serial no. 23. The embryo measured 10 mm. (crown-rump measurement) and was cut into cross-sections of 35 μ thickness. A diagram of the arteries of this series appears in figure 5; a diagram of the veins appears in figure 6. The diagrams show the relation of the vessels studied to the aorta and the postcardinal veins, respectively. The first pair of intersegmental arteries (intercostals of the adult) arise as branches of the axillary; the first pair of intersegmental veins arise from the postcardinals directly. These intersegmental vessels give off branches into the vertebral region and into the lateral body region as they did in the first series studied. The first six pairs of these intersegmental arteries and veins were carefully followed. In the lateral body region it was possible to make out more or less of a capillary plexus established between the intersegmental vessels. As soon as an intersegmental artery reaches the lateral body region, it divides, sending several branches at right angles, some ventrally and some dorsally, to join the branches sent ventrally into that region by the intersegmental veins. The blood carried out into the lateral body region by the intersegmental arteries can be returned in two ways, back by way of the intersegmental veins to the postcardinals or by way of the umbilical vein. Figure 7 is a diagram made from two cross-sections. It shows the relationship between the vessels just discussed. The main point of interest in this series is the distinct advance which has been made in the formation of the capillary plexuses between the intersegmental vessels just discussed.

The third series shows the marginocostal artery almost completely formed. A diagram of the arteries of this series, serial no. 27, will be found in figure 8; a diagram of the veins will be found in figure 9. The embryo measured 15 mm. (crown-rump) and was cut into cross-sections of $35\ \mu$ thickness. Anteriorly the vertebral artery arises as in the adult, as a branch of the axillary. The axillary also, it will be observed, gives off another

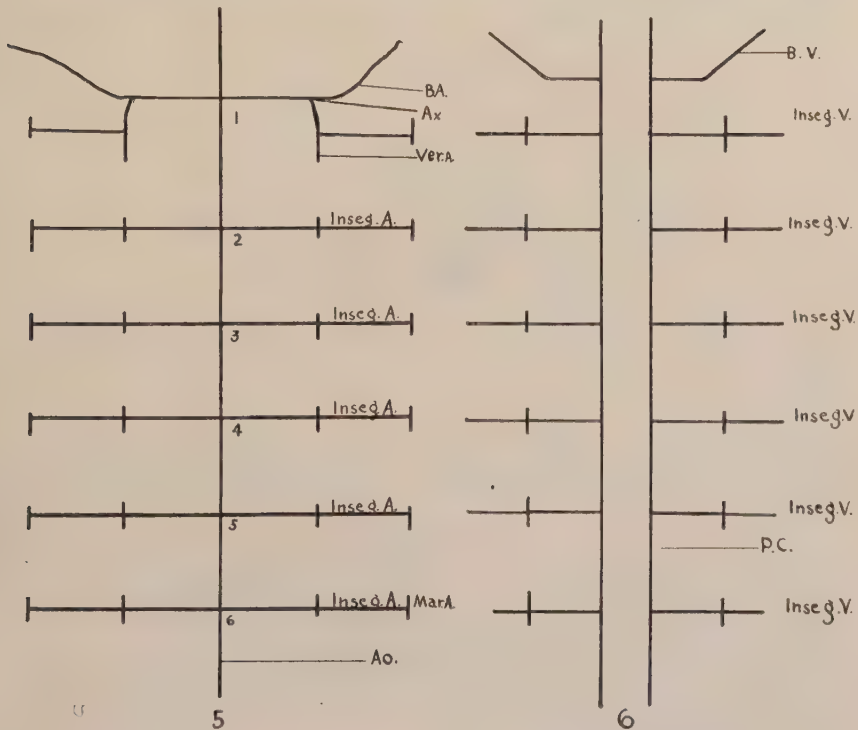


Fig. 5 The arteries. Serial no. 23.

ABBREVIATIONS

- | | |
|---|---|
| <i>Ao.</i> , aorta | <i>Mar.A.</i> , marginocostal artery (beginning of) |
| <i>B.A.</i> , brachial artery | <i>Ver.A.</i> , vertebral artery (beginning of) |
| <i>Inseg.A.</i> , intersegmental arteries | |

Fig. 6 The veins. Serial no. 23.

ABBREVIATIONS

- | | |
|-----------------------------|--|
| <i>B.V.</i> , brachial vein | <i>Inseg.V.</i> , intersegmental veins |
| <i>P.C.</i> , postcardinal | |

branch into the lateral body region, namely, the marginocostal artery. The vertebral artery can be traced as a definite vessel almost to the middle of the embryo. The vessels (old intersegmental vessels) connecting it with the aorta in the anterior region have been lost, but they can still be observed in the posterior region. The marginocostal arteries are fairly well formed. In several places there still seems to be a capillary plexus. This is indicated in the diagram.

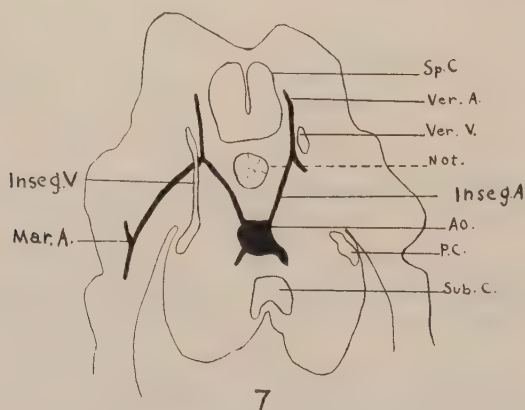


Fig. 7 A diagram of a section through the kidneys at the juncture of the sub-cardinals.

ABBREVIATIONS

<i>Ao.</i> , aorta	<i>Sp.C.</i> , spinal cord
<i>Inseg.A.</i> , intersegmental arteries	<i>Sub.C.</i> , subcardinals (juncture of)
<i>Inseg.V.</i> , intersegmental veins	<i>Ver.A.</i> , vertebral artery (point of formation)
<i>Mar.A.</i> , marginocostal artery (point of formation)	<i>Ver.V.</i> , vertebral vein (point of formation)
<i>Not.</i> , notochord	
<i>P.C.</i> , postcardinals	

The diagram of the veins of this series shows that the post-cardinals are still prominent vessels. Their anterior connection with the sinus venosus has been lost. The first pair of intersegmental veins has also lost their connection with the post-cardinals. The second and following pairs of intersegmental vessels, with a few exceptions, still join the postcardinals. The second pair, however, shows signs of degeneration. Numerous irregularities occur. The right intersegmental vein of the 3rd

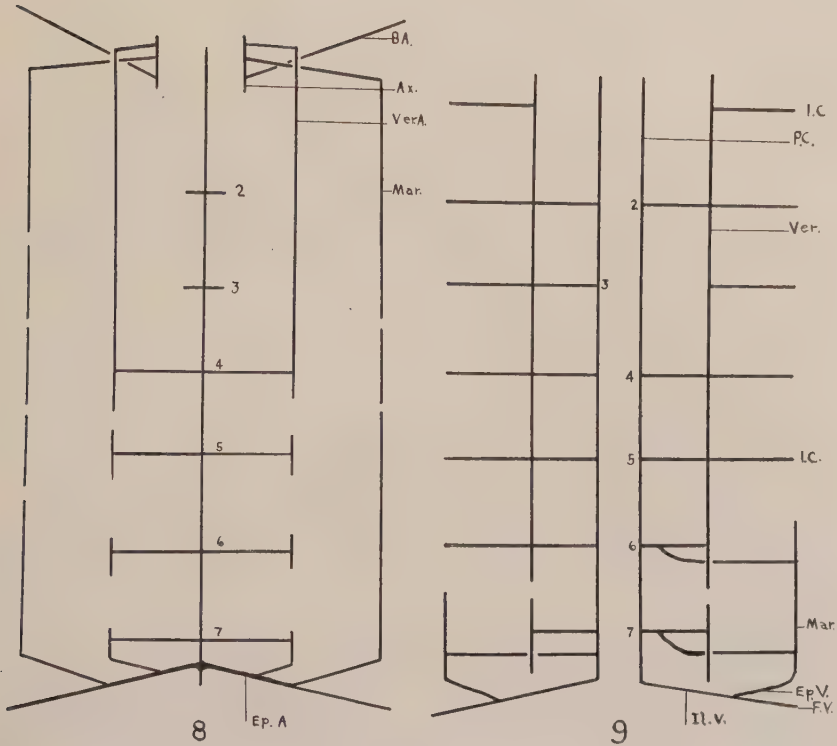


Fig. 8 The arteries. Serial no. 27.

ABBREVIATIONS

Ax., axillary artery
B.A., brachial artery
Ep.A., epigastric artery

Mar., marginocostal artery
Ver.A., vertebral artery
 2 to 7, intersegmental arteries

Fig. 9 The veins. Serial no. 27.

ABBREVIATIONS

Ep.V., epigastric vein
F.V., femoral vein
I.C., intercostal veins
Il.V., iliac vein

Mar., marginocostal vein
P.C., postcardinal
Ver., vertebral vein
 2 to 7, intersegmental connections with
 the postcardinals

pair has lost its connection with the postcardinal; the left one has not. On the left the 6th intersegmental vein comes off the vertebral; on the right the 6th intersegmental vein and the vertebral come off a joint vessel. On the left, the 7th intersegmental and the vertebral come off as separate vessels; on the right they come off a joint vessel. The iliac veins, continuations of the postcardinals, give off the epigastrics just back of ribs VIII. The epigastric joins the marginocostal just anterior to ribs VIII. The marginocostal vein can only be traced forward a short distance as a definite vessel. Anterior to this point it is still in the capillary plexus stage.

The intercostal arteries and veins of this series anastomose freely outside the muscle layer. They form a plexus extending from the marginocostal artery to the vertebral vein. It is easy to see that the important vessels of this series, concerned with the blood supply to the carapace, are the rapidly forming marginocostal arteries which carry most of the blood out into that region and the vertebral veins whose branches pick up the blood from the branches of the marginocostal arteries and return it to the postcardinals.

A diagram of the vessels as they are found in the fourth series will be found in figures 10 and 11. This series is listed as serial no. 20. It measured 20 mm. (crown-rump) and was cut into sections of 35 μ thickness. The diagram shows the marginocostals and the vertebral arteries given off as separate branches of the axillary. The marginocostal arteries are continuous vessels in this series. Posteriorly, they join the epigastric artery, a direct branch of the aorta. The vertebral artery is also continuous. Posterior to rib VIII it sends down a branch which joins the epigastric.

Upon examination of the diagram of the veins of this series, it will immediately be seen that the anterior portions of the postcardinals are rapidly disappearing. They have degenerated as far back as the level of ribs IV. The vertebral vein can be traced as a definite vessel as far forward as ribs II. The connections between the vertebrals and the postcardinals have all disappeared as far back as the level of ribs IV. At this point the

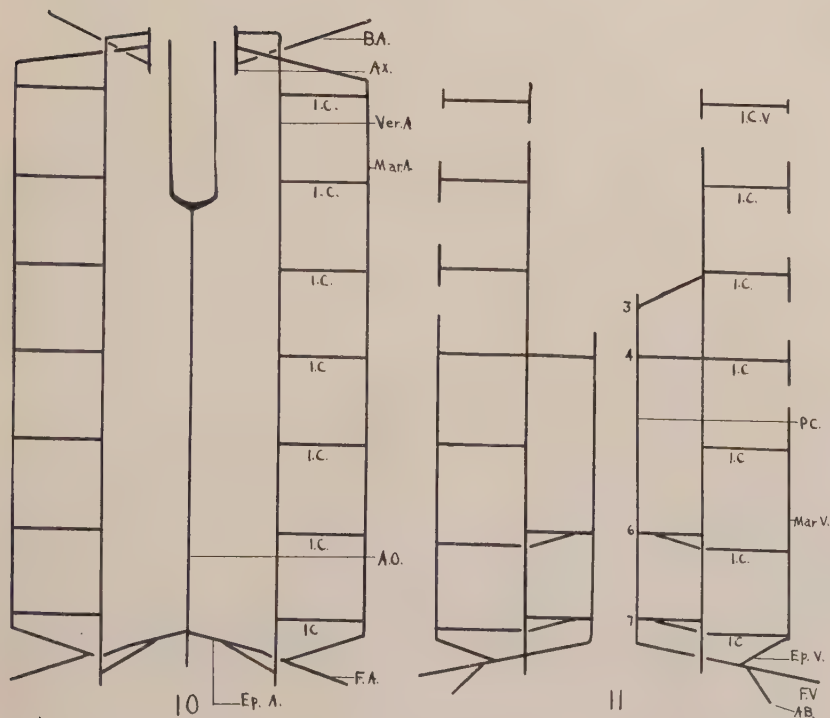


Fig. 10 The arteries. Serial no. 20.

ABBREVIATIONS

A., aorta
Ax., axillary artery
B.A., brachial artery
Ep.A., epigastric artery

F.A., femoral artery
I.C., intercostal arteries
Mar.A., marginocostal artery
Ver.A., vertebral artery

Fig. 11 The veins. Serial no. 20.

ABBREVIATIONS

A.B., abdominal vein
Ep.V., epigastric vein
F.V., femoral vein
I.C.V., intercostal vein

Mar.V., marginocostal vein
P.C., postcardinal vein
 3 to 7, intersegmental connections with postcardinals

3rd intersegmental, on the right side, is seen emptying into the postcardinal. The corresponding vessel on the left side has lost its connection. The 4th pair of intersegmentals retain their connection with the postcardinals. The 5th pair of intersegmentals appear to have degenerated. This is an extremely unusual feature, because it is this pair which is permanent in the adult. All the rest in front and behind lose their connections. Either the connection will be established later or more probably the 4th or the 6th will take over the 5th's function. The 6th pair of intersegmentals, as soon as they come off the postcardinals, divide into two branches. One runs up to join the vertebral, the other runs out into the lateral body region, forming the 6th intercostal of the adult. The 7th pair are similar to the 6th. The iliac, a posterior continuation of the renal portal (postcardinal), divides into three branches just posterior to ribs VIII. These branches are: the epigastric, the circumflex iliac, and the femoral. The marginocostal, a forward continuation of the epigastric, can be traced as far forward as the level of rib V on the right side and rib IV on the left side. Anterior to this, numerous branches are sent down from the vertebral vein. These form a capillary plexus between the ribs out in the lateral body region. It can be said that in this region the marginocostal is still in the capillary plexus stage.

A diagram of the arteries and veins of the 5th series, serial no. 60, will be found in figures 12 and 13, respectively. This embryo measured 23 mm. (crown-rump). Sections of 50 μ thickness were made of it. It will be observed that the brachial, the vertebral, and the marginocostal arteries arise as branches of the axillary. The vertebral artery is continuous throughout its course. It comes off the axillary artery, rises to the position, which it occupies in the adult just anterior to ribs I. Posteriorly it sends down a branch which joins the epigastric between ribs VIII and IX.

It will be noted that the postcardinal veins have practically reached their adult condition. The posterior portions of the postcardinal remain throughout the life of the individual as the renal portals. On the right side the 4th intersegmental vein still

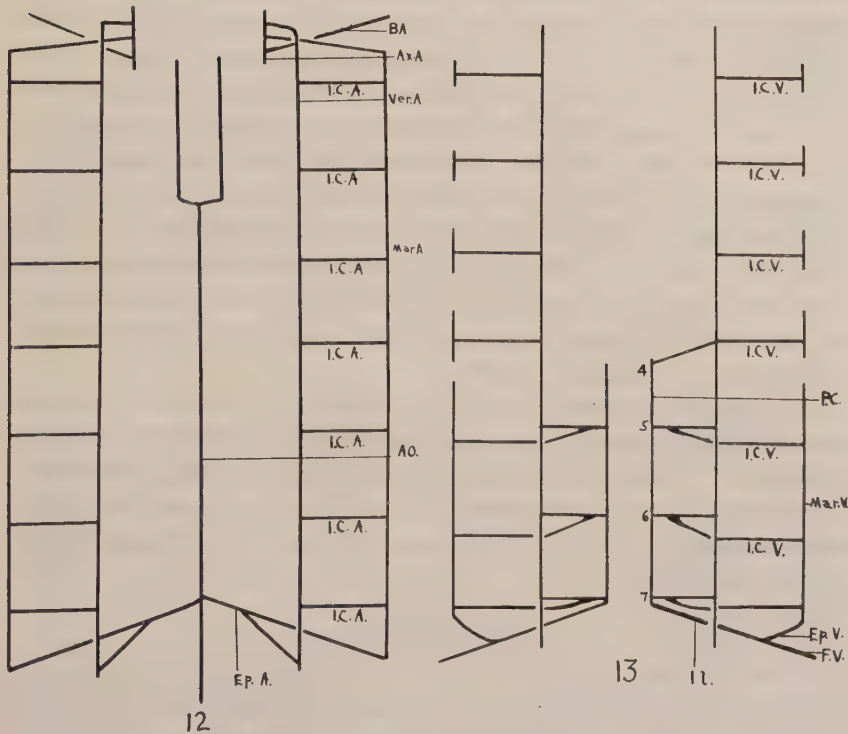


Fig. 12 The arteries. Serial no. 60.

ABBREVIATIONS

- | | |
|--------------------------------|--------------------------------------|
| <i>Ao.</i> , aorta | <i>Ep.A.</i> , epigastric artery |
| <i>Ax.A.</i> , axillary artery | <i>I.C.A.</i> , intercostal arteries |
| <i>B.A.</i> , brachial artery | <i>Mar.A.</i> , marginocostal artery |
| | <i>Ver.A.</i> , vertebral artery |

Fig. 13 The veins. Serial no. 60.

ABBREVIATIONS

- | | |
|-----------------------------------|------------------------------------|
| <i>Ep.V.</i> , epigastric vein | <i>Il.</i> , iliac vein |
| <i>F.V.</i> , femoral vein | <i>Mar.V.</i> , marginocostal vein |
| <i>I.C.V.</i> , intercostal veins | <i>P.C.</i> , postcardinals |
| | 4 to 7, intersegmental connections |

connects the vertebral with the postcardinal. As soon as this degenerates, the renal portals will have reached the climax of their development. On the left side the adult condition has been reached. The 5th, 6th, and 7th intersegmental (intercostals) of this series are all similar—the postcardinal gives off a branch, this branch immediately divides into two vessels, one runs up to join the vertebral and the other runs out into the lateral body region. The latter form the intercostals of the adult.

The marginocostal vein can be traced forward as far as the level of ribs V on both sides as a very prominent vessel. Anterior to this point, the vessel is similar to the preceding stage, but it shows that more of an advance has been made toward the formation of a definite vessel.

An embryo listed as serial no. 8 (uninjected) of about the same size as serial 60 shows the marginocostal veins completely formed. Anteriorly it joins and is continuous with the first intercostal. The renal portals of this series, however, are not so far developed as in serial 60. The 4th intersegmentals on both sides still join the postcardinals.

6. DISCUSSION

A long discussion will not be necessary, since the history of the work which has been done previously upon the blood vessels in question has been discussed under section 3 and since their embryology has been traced out step by step in section 5.

The formation of the marginocostal arteries and veins in turtles has been found to be relatively simple. In pig it is evidently more complex, for Smith has described the formation of four vessels, the thoracoepigastric, the superficial epigastric, the internal mammary, and the deep epigastric, which function in draining the body wall. The first two are outside the muscle layer and the last two are inside the muscle layer. Since mammals nourish their young with milk and since these vessels which Smith describes are closely related to the milk glands, one would naturally expect a more complicated vascular system in mammals than in turtles. In turtles there is only one artery and one vein developed out in the lateral body region.

Several hypotheses might be offered for the formation of the vertebral and marginocostal vessels. Lewis's diagrams of the external mammary vein of rabbits suggested one hypothesis, namely, that the external mammary vein sprouts from the axillary vein, gradually grows backward, and finally joins the femoral. The results of this paper prove that this hypothesis, as far as turtles are concerned, is incorrect.

Kampmeier's paper is of interest from a comparative standpoint. In figure 2 he has shown thirteen pairs of intersegmental veins between the sinus venosus and the iliac veins. In turtle there are only seven or possibly eight (serial no. 40, section 5). In *Anura* all of the intersegmental veins except the 9th and the 11th degenerate. The 9th carry the blood from the dorsolumbar veins (the anterior portions of the old lateral-line veins) to the kidneys. The 11th carry the blood from the posterior vertebral veins (posterior portions of the old lateral-line vein) to the transverse iliac veins. In turtles the 5th, 6th, 7th, and sometimes the 4th intersegmentals remain as the intercostal veins of the adult. This would seem to indicate that the turtles are closely related to the 'lower amphibia,' for Kampmeier says: "Only in the lower amphibia do the intersegmental veins persist as such during the entire life of the animal."

Stromsten ('05) gives a detailed account of the formation of the renal portal veins in a different species of turtle. The results of this paper confirm his work. It has been the purpose of this paper to point out more specifically just how much of the postcardinals are retained. The intersegmental veins have been used as an index in determining the exact amount. It was found that the postcardinals degenerate as far back as the point where the 5th intercostals and the pair of branches sent down from the vertebrals just posterior to ribs V join.

It is of interest to point out that while growth in vertebrates usually proceeds caudally, the vertebral and marginocostal veins do not follow this plan in their formation. Beginning with serial no. 27 and studying through serials nos. 20, 60, and 8, one finds that these vessels begin to form caudally first and gradually connect up with the intersegmental veins anteriorly. Since the

main venous channels lead from the posterior region, it is to be expected that the flow of blood in the superficial vessels should be directed toward these vessels. It is also to be expected that the intersegmental veins nearer the future outlet of this blood should begin to form a continuous channel first and later connect with the more anterior intersegmental veins.

7. SUMMARY

The steps in the development of the marginocostal arteries and veins, the vertebral arteries and veins, the intercostal arteries and veins, and the renal portal veins have been followed through a series of embryos ranging in size from 9 mm. to 23 mm. (crown-rump).

The marginocostal arteries arise by a longitudinal anastomosis out in the lateral body region of the branches sent out into that region by the intersegmental arteries. The marginocostal veins arise by a longitudinal anastomosis out in the lateral body region of the branches sent out into that region by the intersegmental veins.

The vertebral arteries are formed by a longitudinal anastomosis midway and lateral to the spinal cord of the branches sent dorsally into that region by the intersegmental arteries. The vertebral veins are formed by a longitudinal anastomosis ventral and lateral to the vertebral artery of the branches of the intersegmental veins.

The intercostal arteries and veins are derived from plexuses formed by the branches themselves which are sent out into the lateral body region from the intersegmental arteries and veins, respectively.

The renal portal veins of the adult are the posterior portions of the postcardinals, the postcardinals having degenerated anteriorly as far back as the point where the vertebrals send down a branch on either side to join the renal portals posterior to ribs V.

The vertebral arteries lose all of their connections with the aorta except a pair anterior to ribs I. A pair of connections posterior to ribs VIII join them with the epigastric arteries shortly after the epigastrics leave the aorta.

The vertebral veins lose all of their connections with the renal portals except the pair posterior to ribs V.

Seven intercostal arteries join the vertebral artery with the marginocostal artery on either side. Four intercostal veins join the vertebral vein with the marginocostal vein anteriorly. Posteriorly the 5th, 6th, and 7th intercostal veins join the marginocostal with the renal portal vein on either side. These are the old intersegmental branches sent out into the lateral body region from the intersegmental veins.

It gives me great pleasure to express my thanks to Prof. Frank A. Stromsten for his helpful suggestions and criticism of this paper. I wish also to thank Prof. Albert Kuntz and Prof. H. M. Kelly for their helpful suggestions. The work for the most part was done in the laboratories of Animal Biology of the State University of Iowa. I wish here to express my thanks to Prof. Gilbert L. Houser, the director of these Laboratories.

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THE DUCTUS VENOSUS IN THE FETUS AND IN THE ADULT

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TWO FIGURES

Text-books state that the ductus venosus of the fetus, following its postnatal occlusion, persists through adult life as a fine fibrous cord termed the ligamentum venosum. "The umbilical vein and the ductus venosus become completely obliterated between the second and fifth days after birth, and ultimately dwindle to fibrous cords, the former becoming the round ligament of the liver, and the latter the fibrous cord, which in the adult may be traced along the fissure of the ductus venosus" (Gray's Anatomy).

The ductus venosus is a fetal blood vessel which has been formed secondarily by a longitudinal anastomosis of capillary sinusoids of the liver during the early weeks of intra-uterine life, and which, in the mature fetus, as a direct continuation of the umbilical vein, conducts a large part of the placental blood into the left hepatic vein just before its union with the inferior vena cava, thus short-circuiting the liver. The purpose of this short cut during fetal life is to permit the richly oxygenated, placental blood to pass rapidly to the body of the fetus, through which it may be distributed after only slight previous admixture with the hepatic and somatic circulations. The size of the liver, relative to that of the entire body, is much greater during early, than during late fetal life. This is attributable to the fact that all the placental blood must pass through the liver sinusoids up to the time of formation of the ductus, and only a small part after its formation.

The literature on the subject of the ductus venosus is limited to the abbreviated statements found in current anatomical, embryological, and obstetrical text-books and articles scattered

over the last seventy years of medical writing. In 1856, Meadows reported an instance of fatal hemorrhage from the ductus venosus occurring in a child twenty hours after birth, although the evidence on which he localized the lesion to the ductus is not clear. He says: "But on examining the liver an enormous clot was found lying on its upper surface, between it and the peritoneum which had been stripped off by the effused blood; the same thing was also seen on the under surface of the right lobe; these two clots were much thicker behind than before, and seemed to have originated from the ductus venosus; but how the blood got into the cavity of the peritoneum could not be positively ascertained." No cause for the accident could be allocated. There was no history of intrapartum or postpartum injury to the child. The labor and the first twenty hours of extra-uterine life were normal.

Jackson, as early as 1859, mentions the error of most text-books of anatomy, including the volume 'recently produced by Gray,' in which it is said that the ductus venosus enters into the vena cava. According to Jackson's own observations on dissections, "it terminates in one of the hepatic veins near and sometimes close to the opening of this last into the vena cava." He said that Mr. John Bell, in his anatomy (1802) was one of the few previous writers who had formulated this fact correctly. Also in a small volume by Mr. John Struthers of Edinburgh ('54) it was noted that the ductus venosus "enters not the vena cava but the left hepatic vein, about a quarter of an inch before the latter ends in the vena cava." Jackson says that the present instance is one of too many in which errors are perpetuated in the text-books of medical science.

The interesting anomaly of absence of the ductus venosus with a description of the consequences and a summary of four other reported cases is recorded by Paltauf in 1888. The condition is very rare, Küster reporting one case and Otto three cases. Küster's case was complicated by the presence of abdominal fissure, and Otto's showed unusual points of entry of the umbilical vein. Paltauf's was a normal, full-term child, except that such a huge ascites was present that it formed an obstruction to the natural course of labor, and paracentesis had to be resorted

to. Moreover, hydramnios had been present. The placenta was very large, the umbilical cord long, its veins tortuous and showing varicose dilatations; the subperitoneal veins were very large and somewhat tortuous. The umbilical vein led obliquely into the portal vein. No sign, even such as a fibrous strand, remained of the ductus. Thus a condition of portal obstruction in the fetus arose, analogous to the portal cirrhosis of the adult, with a resulting hydramnios, ascites, and attempts at collateral circulation. In this instance prominent anastomoses had been produced on either side between the spermatic vein, emptying into the vena cava, and the colic veins, emptying into the portal vein, and further anastomoses with veins of the abdominal wall. This is a type of collateral circulation unknown in acquired portal obstruction, since in the adult the portal and caval circulations are too widely separated, and the venous channels are no longer patulous, as during the process of development. In explaining this absence of the ductus Paltauf invokes the statement of His, that the ductus venosus is a secondary formation, a short cut of the capillary sinusoids of the liver among the islands of liver cells. In this case such a channel has not been opened up, with the result that all the placental blood must pass through the capillaries of the liver and hence must be under increased pressure.

As to the chief function of the ductus venosus, Paltauf believes that it serves to maintain a free passage for the blood in the umbilical and portal veins, equalizing rapidly the differences in pressure which probably prevail in the two channels, and avoiding any detrimental influence of the umbilical venous flow, which is under greater pressure, on the portal flow. The liver sinusoids of the late fetus, even as the embryonic liver sinusoids, should suffice for the passage of the umbilical blood, provided no secondary disturbances arise. Instances of hydrops and ascites with hydramnios in cases of fetal hepatitis, in spite of the presence of the ductus venosus, serve only to emphasize the prominent part of the sinusoidal circulation even in fetal life.

Barge asks why the ductus venosus closes after birth. It is natural enough that the umbilical vein should become obliterated after ligation of the cord, for blood vessels usually close distal to

a ligature. The ductus has been regarded as a continuation of the umbilical vein—a point which would afford a simple explanation were it not for the fact that the portion of the umbilical vein, known in the adult as the recessus umbilicalis of the portal vein, persists and functions throughout life. In this case the circulation disobeys the law of hydrodynamics which would have it flow in the line of least resistance, namely, through the ductus; but after ligation of the umbilical vein it chooses to pass through the sinusoids of the liver where it meets much more resistance. This raises two questions: Is it not possible that the ductus closes during fetal life? There is no evidence to support this view, apart from Gegenbauer's observation that the lumen of the duct gets narrower during fetal life. Is the closing of the duct a useful occurrence? Surely it is, for it does not seem practical that a large volume of blood should dodge the liver, one of the main organs of general metabolism. But neither this practical side, nor the hereditary tendency defended by Roux, explains the mechanism of closure of the duct. Barge emphasizes the initiation of respiration after birth and the cessation of the fetal circulation, especially the former. During inspiration the sinusoids of the liver are compressed by the diaphragm, while during expiration the pressure lets up and a kind of suction action then draws the main part of the blood into the liver, resulting in a reduction of the volume of blood in the ductus which furthers the closing of the ductus. DeVries objects to this hypothesis on the ground that respiration is too rapid a process to permit the endothelial lining of the duct to stick together long enough to result in a 'growing together' of the duct walls. Bolk attributes the collapse of the ductus to the negative venous pressure arising after birth. Boeke has found the ductus collapsed without obliteration in several instances among older children.

Richter says that the ductus venosus passes through the fissure between the Spigelian lobe and the left lobe of the liver, from the juncture of the umbilical vein with the left branch of the portal vein, to the left branch of the hepatic vein immediately before it empties into the inferior vena cava. During the first week of life the ductus can be injected, and shows a lumen of 1 to 2 mm.

in diameter. Closure begins at the portal end of the ductus at the end of the first, or during the second week. By the fourth to the sixth week it is completely obliterated except for a small pit at the hepatic end. Although the lumen is obliterated, one often finds endothelial-lined spaces filled with blood in the connective tissue filling the original lumen, and shoots of vessels grown through the original vessel wall into the connective tissue of the lumen and supplying it. In the process of closure the incompletely obliterated lumen is in part distended with thrombi which are in process of being replaced by a proliferating network of fibers. In some instances openings of the lumen of the ductus may occur, and even communications with portal branches, which may form a basis for later communication between the portal vein and the vena cava in cases of cirrhosis of the liver.

EMBRYOLOGY

The first paired vessels of human embryos (according to Prentiss) are formed as longitudinal anastomoses of capillary networks which originate first in the angioblast of the yolk-sac and chorion. In embryos of 1.3 mm. umbilical veins from the chorion, fused in the body-stalk and separating to pass forward in the somatopleure on either side to the paired tubular heart, are already formed. At 2.5 mm. a longitudinal fusion of the paired heart has occurred, and numerous tributary veins from the yolk sac, united into a single vessel on either side, join the umbilical veins to empty into the sinus venosus, one of the three primitive dilatations of the heart tube, at its base. At 4.2 mm. the umbilical vein on either side has been joined by the common cardinal vein, the resultant trunk entering the sinus laterally. At this stage the liver diverticulum has grown out ventrally from the foregut cranial to the wall of the yolk-sac, and is beginning to grow into the walls of the vitelline veins, producing minute isolated islands of liver cells surrounded by the vitelline sinusoids. Between the liver diverticulum and the yolk sac three transverse anastomoses between the vitelline veins, one dorsal and two ventral to the primitive gut, arise. Shortly after this an oblique venous passage is opened up among the islands of liver cells,

passing from the juncture of the left vitelline and umbilical vein (for the latter has now approached and tapped the former on each side) forward to the point of entry of the right vitelline vein into the sinus venosus. This secondary formation and short cut for the main bulk of the placental blood, which now enters largely through the left umbilical vein (the proximal end of this vessel and its union with the sinus venosus disappearing), becomes the ductus venosus. The vitelline veins distal to the liver, through the atrophy of the parts of the two venous rings encircling the gut and the growth of others, come to form the portal vein of the adult.

THE POSTNATAL DUCTUS VENOSUS

The present study is based on a survey of material removed at fifty consecutive postmortem examinations at the Mayo Clinic. In one case, that of a still-born child at full term (case A400979) the ductus venosus was completely patent, although the first signs of closure were evident. There was a sharp line of demarcation between the intima of the umbilical vein and that of the ductus, the two being of notably different shades of color. The circumference of the ductus at the end adjoining the portal vein was much less than that at the end adjoining the left hepatic vein, 7 mm. as compared with 12 mm. The circumference of the left branch of the portal vein at its point of union with the umbilical vein was only 3.5 mm.—a fact which would lead one to suspect that twice as much of the umbilical blood flowed through the ductus as did through the circuitous route of the liver capillaries. The umbilical vein, too, was completely patent and showed no narrowing, although the intima distal to the point of entry of the veins from the quadrate lobe and the left lobe of the liver (the future recessus umbilicalis of the portal vein) showed distinct roughening and absence of the normal intimal sheen.

In one child of eleven hours (case A398634) there were no signs of closure, or of narrowing of the ductus, except that the same sharp line of demarcation existed between the intima of the umbilical vein and the ductus as in the still-born child.

In another child, who had lived two days (case A401005), there was no opening or even a trace of a scar indicating an opening at the umbilical end (now the portal end) of the ductus, although the hepatic end was widely open, as in the fetus, and the duct itself was freely patent almost to its point of union with the portal vein. Several small veins which drained the surrounding liver tissue emptied into the hepatic half of the ductus, the embryologic channel thus serving also as a hepatic vein. The umbilical vein was completely closed except for a minute pinhole opening into the recessus umbilicalis and a channel of similar size present in the round ligament.

In a case of congenital heart disease with pulmonary atresia in a child of four and one-half months (case A395079), the ductus was completely obliterated with neither opening nor scar at either end.

In a girl of thirteen years (case A400834) a ductus patent in its hepatic half, with an opening 2 mm. in diameter into the hepatic vein, was found. There was no sign of the portal end of the ductus. I injected India ink into the patent end of the ductus and the ink passed out into and distended certain small hepatic veins draining the left lobe of the liver, small vessels running in the hepatogastric ligament, and the vasa vasorum of the ductus itself. In the open part of the ductus five small veins were seen to enter its lumen, through which the injection-mass had passed. The portal half of the ductus did not take the India ink.

In an analysis of the cases seen in adult life, the most striking fact is that there persists a definite, although often semiobliterated duct, lying close to the liver in the fossa for the ductus venosus. It appears as a scant bundle of fibrous strands, semitranslucent, yet denser than the thin fibrous sheet, the hepatogastric ligament, near the bottom of whose meshes it extends from portal to hepatic vein. It is extremely difficult to locate. But in the center of this apparently insignificant, flat, fibrous band there may be traced in each case a definite duct, often intima-lined and glistening, especially in its hepatic half, even though there is no connection with the veins at either end. In a few instances such a complete patency is shown through the whole length,

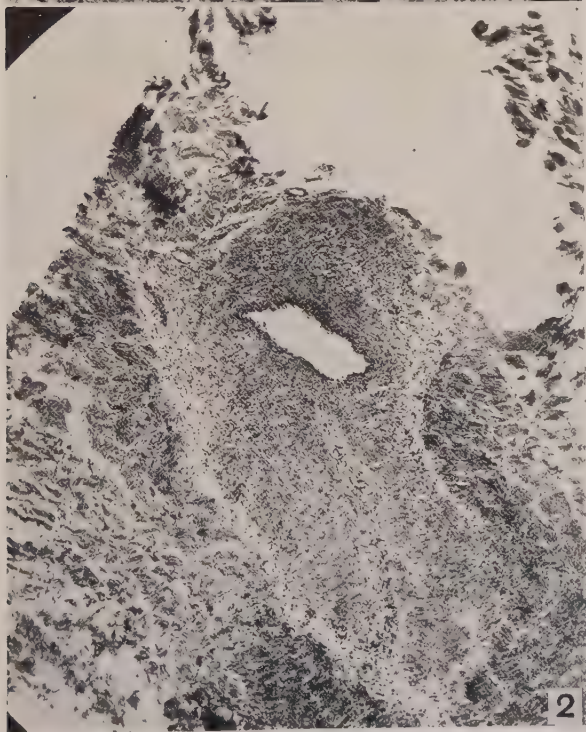
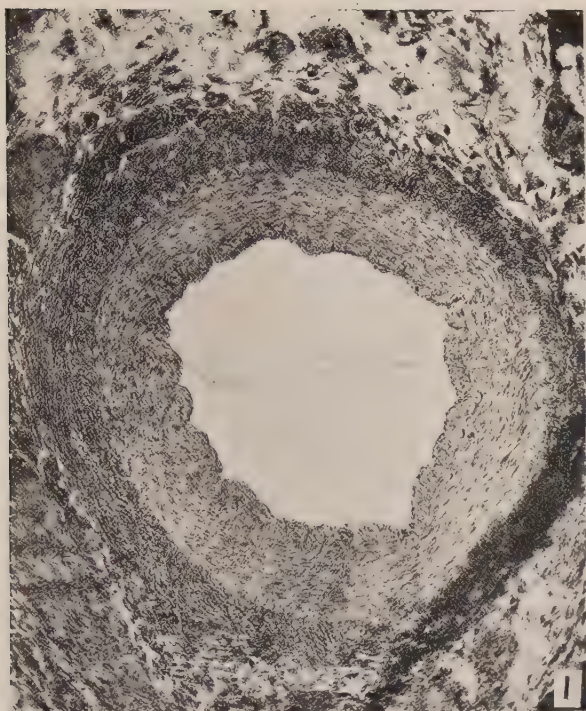
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but more often it is only near the hepatic end. The remainder of the ductus may present a lumen after a passage has been gently forced by a probe. But in every case a pathway which once represented the lumen of the ductus venosus may be dissected out grossly. Microscopic examination confirms this, for the entirely patent sections present a vein of normal appearance, a thick wall with all its coats, and a large circular lumen (fig. 1). The sections from the semiobliterated zones show a minute, circular, or a flat, elongated lumen, endothelium-lined in some places, and with rough, fibrous elevations or irregular projections from the intima at others (fig. 2). In no case was an open passage persistent from the portal vein to the hepatic vein. It is interesting to speculate as to what clinical syndrome, if any, a ductus venosus, completely patent throughout adult life, would give. Certainly it would lead to some metabolic disorder, possibly to a condition similar to that resulting from an Eck's fistula. It is worthy of note that a patent ductus is not found as one of the channels of collateral circulation in cases of secondary portal obstruction, such as cirrhosis of the liver.

The duct ranged between 35 and 65 mm. in length in the adult cases, the average being 45 mm. The livers from these weighed between 825 and 2600 grams, with an average weight of 1680 grams. There seemed to be no correlation between the length of the ductus and the weight of the liver. In the portal vein at the end of the ductus there was almost invariably a smooth intimal lining showing no opening into the ductus and no pit or scar to mark the site of the former opening, except that by traction on the ductus the intima could be depressed, revealing its former location. One case presented a minute depression. Another showed a minute, pinhole opening leading into the ductus, which was widely patent down to within 1 cm. of its hepatic end, where it had closed without leaving a scar.

Fig. 1 (A401367.) Patient aged thirty-three. Section through hepatic end of ductus venosus. Both openings closed without scars. $\times 50$.

Fig. 2. (A397718.) Patient aged seventy-one. Section through middle of ductus venosus. Both openings closed without scars. $\times 50$.



In fifteen cases the hepatic vein presented an opening into the ductus, varying from the size of a pin-point to 2 mm. in diameter. The lumen of the ductus was either definitely patent, or could be forced open by the probe in most of these cases. In a few instances an oval thinning of the intima of the hepatic vein was evident; in a few there were fatty changes in this intima; in one, a narrow, fibrous, intima-covered bridge, 5 mm. long, across the closed oval, and in one, two circular depressions in the intima, side by side, partly covered over by a thin sheet of intima. The location of this hepatic end of the ductus is quite constant, usually just above, occasionally beside the juncture of the upper and lower branches of the left hepatic vein, this common left hepatic vein being a very short vessel which empties almost at once into the inferior vena cava. The portal end of the ductus lies in the upper part of the left main branch of the portal vein, directly opposite to, or a little to the left of the point of juncture of the round ligament with the processus globularis of the portal vein.

In eight instances a definite opening, in one 7 mm. in circumference, led from the portal vein into a patent channel in the round ligament, usually soon ending in a fibrous obliteration, but in one case of Laennec's cirrhosis, extending as a wide, somewhat tortuous channel through the whole length of the round ligament.

SUMMARY

1. The ductus venosus extends from the left branch of the portal vein, opposite the point of entry of the umbilical vein, in the left posterior fossa of the liver in the folds of the hepatogastric ligament, to the point of union of the upper and lower branches of the left hepatic vein, just before the common trunk empties into the inferior vena cava.

2. The portal end of the ductus venosus closes within the first two days after birth, the hepatic end may present a functioning stoma throughout life. The hepatic half of the ductus venosus may remain open, receive tributaries from the parenchyma of the liver and the hepatogastric ligament, and thus function as a hepatic vein in the adult.

3. In every adult the remains of the fetal ductus venosus persist as a fine, flat, fibrous cord with a lumen only semiobliterated even though both ends are completely closed.

4. No cases of complete patency of the entire ductus in adult life were observed personally or seen recorded in the literature.

5. The ductus venosus is a short cut around the liver for the oxygenated fetal blood in its passage from the placenta to the heart.

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THE PLACENTA OF THE GUINEA BABOON (CYNOCEPHALUS PAPIO, DESMAR.)

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ONE TEXT FIGURE AND FOUR PLATES (ELEVEN FIGURES)

This paper describes the structure of the placenta of the Guinea baboon at four months' pregnancy. The animal died as the result of a fall in the Riverdale Zoological Gardens, Toronto, and was placed in my hands by Dr. J. A. Campbell, veterinary surgeon to the Gardens. I wish to express my thanks to him for bringing me the specimen as soon as possible after death.

The duration of pregnancy was judged from the time of disappearance of color from the ischial patches.

The uterus measured undisturbed in situ 125 mm. in length by 100 mm. dorso-ventrally by 85 mm. from side to side; it was pear-shaped and flattish on the dorsal surface, markedly convex on the ventral.

It was opened by a ventral incision and the amnion was found to be closely applied to the chorion at all points, and this was fused to the uterine wall. There was no decidua capsularis.

The placenta was symmetrically placed on the dorsal wall of the uterus; it was a circular disc 80 mm. in diameter and averaging 10 mm. in thickness; its surface was a uniform dark green in color like that of the 'green border' in various carnivore placentae; the courses of the superficial vessels were very clear. The area of attachment was smaller all round than the extreme diameter by about 8 mm. There was no trace of the second placenta usual in the lower Catarrhines. Round the placenta the uterine wall was thicker than in more remote areas, possibly a condition representing the capsularis incompleta described by Strahl for a number of forms. The umbilical cord was inserted at the center of the placenta at a very oblique angle.

The foetus, measured fresh, had the following dimensions:

Crown to root of tail	122 mm.
Atlanto-sacral length	85 mm.
Thigh	17 mm.
Lower leg	32 mm.
Standing height	171 mm.

The general appearance of the foetus and its adnexae is shown in figures 1 and 2. The specimen compares very closely, as far as the stage of development is concerned, with Schwalbe's figure of *Macacus* (Selenka, Studien, 15, pl. I, fig. 2); this is especially true of the great development of the sinus hairs; over the rest of the body hair roots are visible with a hand lens, but are quite inconspicuous. The nails are well-developed.

About half the placenta was removed and pieces were fixed in Dahlgren's and Spueller's fluids, the rest together with the foetus being preserved in 4 per cent formaldehyde. Figures 1 and 2 show respectively the cut face and the amniotic surface of the placenta and part of the uterine wall; figure 3 illustrates the edge of the placenta enlarged two and one half times.

Even at this low magnification the main features are clearly visible. Externally is a stout muscular layer; this is followed by a well-defined spongiosa, and in turn again by a thick compacta; between the expanded glands of the spongiosa and the substance of the compacta blood vessels can be seen as sharply defined dark spots. Resting on the compacta is the mass of villi forming the bulk of the placenta foetalis. Main villous stems are cut across in many places and anchoring villi can be distinguished, especially in the overhanging part of the placenta. The whole is bounded by the chorion and amnion, the latter in some places lifted clear of the chorion. The marginal overhang is very clear and is caused by the chorion's cutting into the compacta. The vaguely defined dark areas in the maternal tissues mark the sites of masses of extravasated blood, and similar regions among the villi indicate particularly dense clots of blood in the intervillous space. The circular white patch is an anaemic infarct which shows microscopically the same structure as the white infarcts of human placentae.

The general appearance of a section through the margin of the placenta is shown in figure 4. The piece altered in shape during excision and fixation and thus presents an outline somewhat different from that shown by figure 3. The much-branched villi are fairly regularly distributed throughout the intervillous space and for the most part the cut branches are small, though here and there are to be seen conspicuous straight trunks running parallel to each other, very much as has been described for *Hylobates* and *Semnopithecus* in earlier stages of development, stages, however, in which the placenta has already acquired its characteristic form (Selenka, Studien, 12, pp. 459 and 521). Anchoring villi are numerous and appear as small dark protuberances on the basal layer, which is clearly defined and can be seen running up the margin of the placenta inside the chorion. The maternal part calls for no special comment save perhaps as regards the arrangement of the blood vessels at the inner edge of the section. Here they are disposed in a cluster surrounded by a rather clearly specialized area of connective tissue to form a trabecula or septum such as is described for *Semnopithecus* and *Simia satyrus* (Selenka, Studien, 11), though the arrangement here is not as pronounced as in those forms.

Under higher magnification the villi are seen to be covered by a continuous layer of syncytium provided usually with but one row of nuclei (figs. 5 and 6). The cellular layer has completely disappeared as such everywhere, with a possible exception to be mentioned later. This syncytium is continuous over the surface of the basal plate between the points of attachment of the anchoring villi and forms, of course, also the chorionic epithelium between the roots of the villi. Its outer surface shows very generally a bristly appearance, which may indicate the presence of prickly processes, though the structure is much coarser than in man; alternatively it may be a syncytial modification *sui generis*, or possibly it is an expression of incipient maceration, though this does not seem likely. From the syncytium spring numerous syncytial processes projecting freely into the intervillous space; they are generally club-shaped and have a number of nuclei

arranged as a sort of core; their surface shows the same frayed appearance as the surface of the villi.

'Giant cells' are present in scattered areas of sections (fig. 5), and while some may be isolated syncytial masses in the intervillous space, this is by no means always, or even usually, the case, since all those that have been followed through a series of sections have been found to join a villus; they are thus sections of syncytial buds.

The syncytium forms also a peculiar network between the villi. This is unevenly developed, being much more marked in some areas than elsewhere; such a region is shown in figure 5. These intervillous strands are of two forms; the first, and much less usual, is thin cords of cylindrical section; the other consists of wide, flat bands of syncytium sometimes extending through ten or more sections each $10\ \mu$ thick. These peculiar relations were confirmed by a wax reconstruction. Some of the 'giant cells' are probably sections of this syncytial network. An apparently comparable arrangement has been described in the placenta of *Mycetes* (Selenka, *Studien*, 14, p. 505) and as far as I know not elsewhere. It is true that the specimen of *Mycetes* was at a much earlier stage of development, but the characteristic structure of the placenta was well developed, and it does not seem probable that further growth would alter materially the relations of the parts. The occurrence of so striking a syncytial modification in two forms so widely separated within the order is yet another demonstration of the care with which the anatomy of the placenta must be used as a criterion of systematic position, save on the broadest lines.

The stroma of villi cut parallel to the long axis is distinctly fibrillar in appearance, with nuclei much elongated; in transverse section the cells form a loose network (fig. 5). No Hofbauer cells have been seen.

Trophodermic cell islands are widely distributed but by no means abundant; they are nearly all quite small, only two or three times the diameter of an average villus, but occasionally a considerably larger mass occurs. The masses often connect several villi together. The cells of which they are composed resemble in

appearance decidual cells, as has been noted for such structures in the human placenta. These cell islands are in all observed cases completely covered by a layer of syncytium continuous with the syncytium of the villus to which they are attached and having the same characters as this layer. A small mass is shown in figure 6. Near the mass, e. g. at the point marked X, there are a few nuclei lying, each in a more or less clearly defined cell-territory of cytoplasm; the nuclei themselves are rounder than those of the syncytium, and it seems likely that they are a remnant of the cellular layer. Similar cells have been found near other trophodermic cell islands.

The center of the masses is frequently necrotic and consists in sections of an area staining purple with Ehrlich's haematoxylin, which passes gradually into the normal cells of the periphery of the mass (fig. 6).

Villi can in some instances be seen to penetrate to a considerable distance into the substance of a cell mass much as in Grosser's well-known figure for the human placenta at an earlier stage of development. Where this occurs there is no epithelial tissue between the stroma and the surrounding cells the tissues being separated by a structureless layer that stains red with acid fuchsin. This layer is continuous with the syncytium at the point at which the villus enters the cell mass.

Anchoring villi present an appearance comparable with that of a cell mass. The syncytium passes uninterruptedly from the villi over the basal plate, the stroma coming into more or less direct contact with the maternal tissues. In nearly all cases the tip of an anchoring villus expands into a mass of cells extremely like those of a trophodermic cell mass, and having the same relations to the stroma. This somewhat club-shaped mass spreads out on the maternal tissue and penetrates it in such a manner that the boundary between the two tissues is entirely obscured to form a chorio-basalis. In places a well marked layer of cells derived from these anchoring masses extends beneath the syncytium over the basalis as a continuous layer between the points of attachment of neighboring villi. These relations are illustrated in figure 7, drawn from a section overstained in iron haematoxylin.

Occasionally the mass of cells fails to become conspicuous at the tip of a villus, and the stroma coming into direct contact with the maternal tissues may burrow some distance into them; at all points it remains quite distinct from them. Figure 8 shows such a villus, which recalls Selenka's drawing for *Hylobates* (Studien, 10, p. 367).

An interesting point is that at the base of the larger villi there are between the stroma and the syncytium masses of cells which look in all respects like those at the two places already described; these tend to spread out between the villi to form a more or less continuous sheet separating the syncytium from the underlying chorionic mesoderm (fig. 9, *Mc*).

The maternal component of the placenta shows the characteristic structures very clearly. The thick muscularis gives way quite suddenly to a layer of very much expanded glands, very obvious in figures 3 and 4. The glands are very irregular in shape and the epithelium lining them is nearly everywhere a flattened layer of degenerating cells, which over wide areas appear to be falling into the lumen to contribute to the detritus often filling it. Only in the deepest regions does the epithelium retain a cubical or columnar aspect over small areas, and all stages between this and the desquamating cells described above can be found. Between the glands run blood vessels passing from the muscularis to the compacta.

The compacta is thick and well-defined, composed for the most part of decidual cells very loosely packed. These cells are not unlike those from the human compacta. Among them are numerous thin-walled blood vessels and a considerable amount of extravasated blood, while an occasional very large vascular trunk passes through to open into the intervillous space.

There is a well-marked layer on the foetal side of the compacta produced apparently by a necrotic modification of the cells of this region. Beyond a rather clearly marked line the decidual cells lose their clear outlines and form a streaky mass in which the nuclei lie, each in its own space; the appearance is very like that of some specimens of cartilage. The matrix stains pink with acid fuchsin. Ill-defined, very irregular areas within

this region stain more deeply, in fact like fibrin, and if this is the true interpretation the layer corresponds very closely to Rohr's layer in the human placenta. On the foetal side this layer passes into the chorio-basalis (fig. 7).

At the overhanging edge of the placenta the chorion cuts deeply into the compacta, dividing it into a maternal segment continuous with the decidua vera, and a foetal segment which together with the chorio-basalis, curves up against the chorion to form a species of closing plate. This plate, to which a number of anchoring villi are attached, thins out rapidly until it consists solely of cells derived from the proliferation of masses at the tips of the villi. This layer is continuous, at least in places, with the subsyncytial sheet originating from the groups of cells at the bases of the larger villi (*Mc* in text figure). The relation of the chorionic epithelium of the decidua vera to the 'closing plate' is interesting. A wedge, as already noted, divides the compacta into two portions; the foetal segment of the compacta extends towards the edge of the placenta between the chorio-basalis and the chorionic wedge. Marginally the chorio-basalis is continuous in the closing plate with the subsyncytial sheet clothing the chorion, and this layer is split by the extension of the compacta at the point marked *Cp* in the text figure. No epithelial layer ordinarily intervenes between the chorionic subsyncytial cell layer and the mesoderm, but at about the marginal limit of the split a cubical epithelium quite suddenly appears and, as the irregular layer of cells thins out and vanishes, becomes closely applied to the wedge of compacta. This epithelium is two cells thick and the layers frequently separate for a short distance to produce small vesicles (fig. 11, *Chv*). Figure 10, which shows these structures on a small scale, should be compared with Selenka, Studien, 13, plate XXVI, the placenta of *Mycetes* at a somewhat more advanced stage of development. Here also the chorion undercuts the disc of the placenta, but in this case it penetrates the spongiosa. The text figure and figures 10 and 11 illustrate these points.

The non-placental part of the uterine wall is of the same structure as the sub-placental wall; the three layers are here of

the same thickness and the compacta is closely covered by the adherent chorionic epithelium.

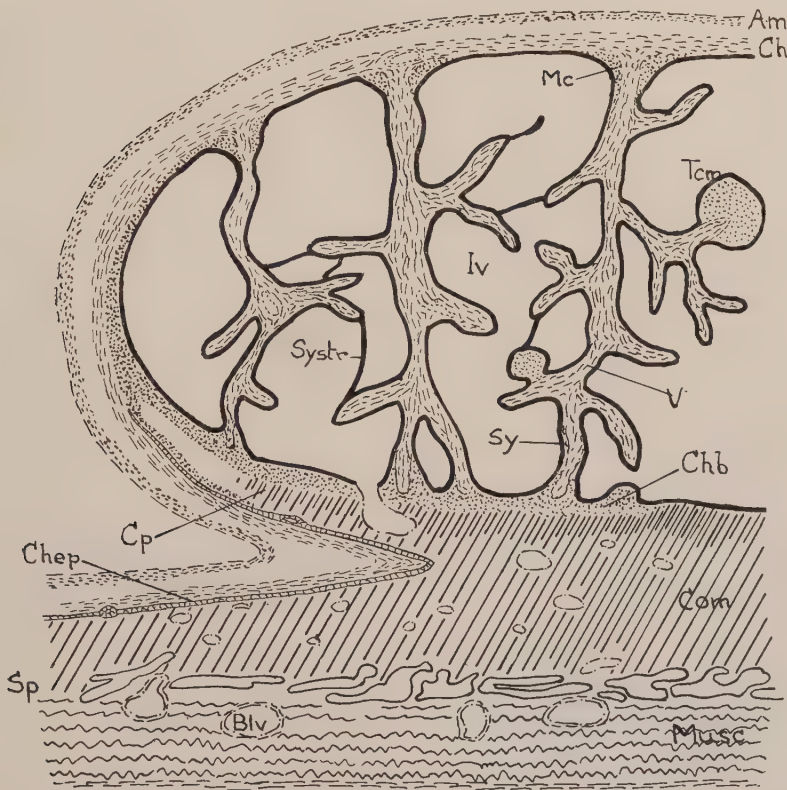
DISCUSSION

The chorion, the villi, the cell masses and the face of the chorio-basalis are all covered with a continuous layer of syncytium, broken only at the points of entry of blood vessels into the intervillous space, which is thus enclosed entirely by a (presumed) foetal tissue. On the side of the syncytium remote from the intervillous space and between it and the underlying tissues at numerous points are patches of cells of very various extent, which over large areas, especially on the basal side, form a continuous layer. It is very tempting, then, to consider that the patches of cells at the bases of the villi, the foetal elements of the chorio-basalis, the sheet of cells in the region of the 'closing plate' and the trophoderm masses are all persistent remnants of a layer originally continuous, in posse at least, beneath the syncytium. Since further there are to be found in the neighborhood of some of the trophoderm cell masses a few cells which seem certainly to be survivors of the layer of Langhans, the suggestion is put forward that the whole of this series of cellular elements is a product of the original cell layer which underlay the syncytium.

In the human placenta the trophoblastic masses are not covered by a continuous layer of syncytium, and it may be doubted whether this speculation is applicable to their origin; it is not, however, hard to imagine the process so modified as to produce the arrangement found there. It is usual to find pieces of syncytium and syncytial buds scattered over the surface of the masses, and it is sometimes possible to discover at the point of insertion of a villus an extension of the syncytium over the surface of the mass. If, then, we imagine the human syncytium to be less plastic, less capable of stretching, we can easily imagine it breaking up into discontinuous patches as the developing mass subjects it to increasing tension.

Grosser, on the other hand, clearly indicates that the villi in man, even in the depths of the cell mass, are covered with a

continuous layer of syncytium, and it is in fact possible to find in the same specimen a villus imbedded in a mass and clearly surrounded by a syncytium and a villus, also imbedded, which retains no trace of the syncytium over the area of contact with the mass.



A slide in this laboratory has a bearing on this point. It is a section of part of a human placenta approximately nine and a half weeks old and shows at one place a medium-sized villous branch adhering to a trophoblastic mass and retaining as a fully continuous layer the cells of Langhans, which separate the stroma of the villus from the cells of the mass; over the area of contact the syncytium has entirely disappeared. This does not,

of course, necessarily disprove the origin of the mass from the cell-layer, since its products might exist along with it, but on the other hand it is no evidence in favour of this view.

It seems probable in the light of this conflicting evidence that the origin of the trophoblastic masses may not be identical in the two cases, and it would be unwise to push the comparison, especially in view of the advanced age of the specimen that forms the subject of these notes.

Whether there is in the human placenta any structure comparable with the continuous layer of syncytium that covers the chorio-basis in the *Cynocephalus*, *Semnopithecus* and *Mycetes* seems to be an open question. Jenkinson states very definitely that the basis is covered not only by a syncytium but in the younger stages by a layer of Langhans cells as well. Frassi describes the face of the maternal tissue as being everywhere covered by a single layer of cells resting almost everywhere directly on the decidua, but, as he thinks, certainly derived from foetal tissues. It passes at the anchoring villi into the syncytium.

Bonnet, on the other side, states as his opinion that the intervillous space is bounded on the maternal side only by decidua or by the angiothelium of the vessels opened by the action of the trophoblast, and Kollmann's drawings uniformly suggest the sudden ending of the syncytium where the villi come into contact with the basis.

Grosser, in Keibel and Mall's *Embryology*, describes basal ectoderm as occurring in clumps of degenerate cells on the maternal side of the intervillous space; these he derives from the covering layer of Frassi.

An examination of available human material having a bearing on the matter reveals that in some specimens considerable areas of the basis are covered by a layer closely resembling, if not identical with, the syncytium of the villi; it is, however, hard to demonstrate that this is really continuous with the epithelium of the villi as convincingly as in the baboon.

To sum up; the placenta of *Cynocephalus* is typically Primate in structure; it cannot be compared in detail with the published

accounts of the structure of the human placenta, though it comes very close to Jenkinson's; the structure of the syncytial network is paralleled in *Mycetes*, but has here marked peculiarities; the relation of the syncytial layer to the trophoblastic cell-masses appears to be unique.

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ABBREVIATIONS

<i>Am.</i> , amnion	<i>Gc.</i> , giant cell
<i>Avi.</i> , anchoring villus	<i>Inf.</i> , infarct
<i>Blv.</i> , blood vessel	<i>Iv.</i> , intervillous space
<i>Ch.</i> , chorion	<i>Lc.</i> , leucocyte
<i>Chb.</i> , chorio-basalis	<i>Mc.</i> , subsyncytial cells
<i>Chep.</i> , chorionic epithelium	<i>Nec.</i> , necrotic tissue
<i>Chv.</i> , vesicles in chorionic epithelium	<i>Sp.</i> , decidua spongiosa
<i>Com.</i> , decidua compacta	<i>Str.</i> , stroma of villus
<i>Cp.</i> , wedge of compacta in 'closing plate'	<i>Sy.</i> , syncytium
<i>Exbl.</i> , extravasated blood	<i>Syst.</i> , syncytial strand in network
<i>Tcm.</i> , trophoblastic cell mass	<i>Vi.</i> , villus

PLATE 1

EXPLANATION OF FIGURES

- 1 Side view of foetus and cut surface of placenta and uterine wall. Photo. $\frac{2}{3}$ nat. size.
- 2 Ventral aspect of foetus and foetal surface of placenta and part of uterine wall. Photo. $\frac{1}{3}$ nat. size.
- 3 Edge of placenta showing amnion, chorion, villi, infarct and maternal tissues. Photo. $\times$ about 2.
- 4 Section of edge of placenta and underlying maternal tissues. Photo. $\times$ about 2.

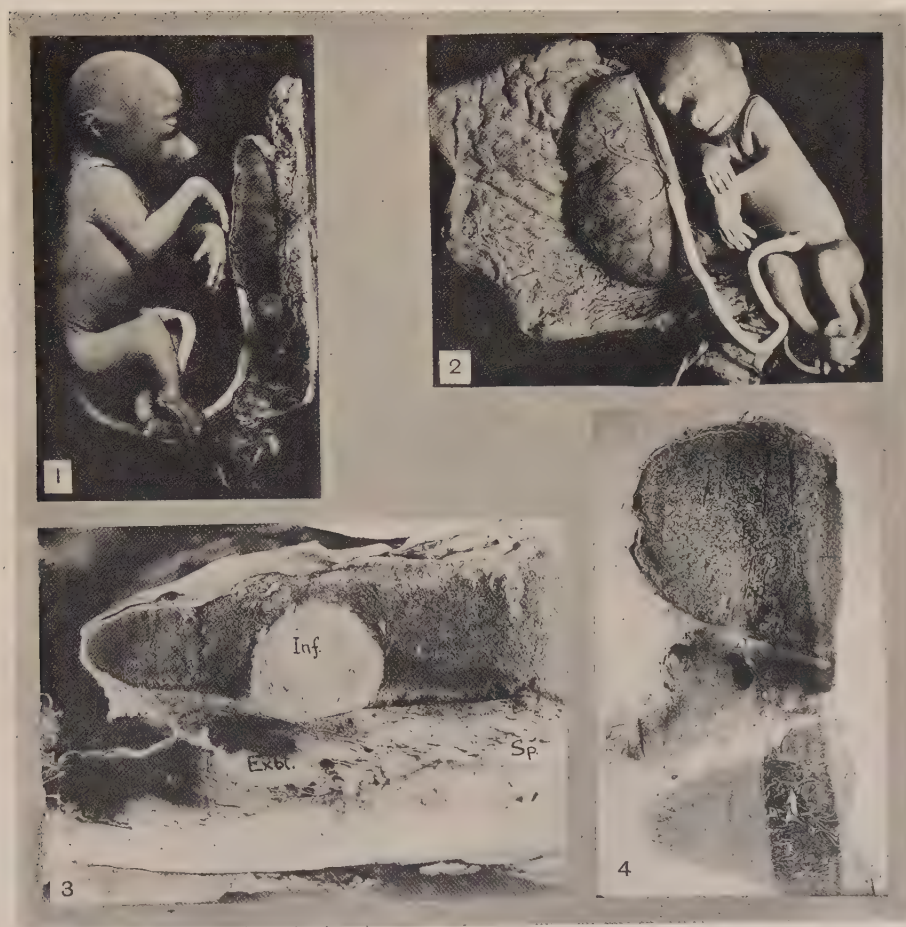


PLATE 2

EXPLANATION OF FIGURES

- 5 Syncytial network between villi. $\times 113$.
- 6 Villus with attached trophoblastic cell mass, showing syncytial covering of the mass and the necrotic interior. $\times 113$.



PLATE 3

EXPLANATION OF FIGURES

- 7 Anchoring villi and necrotic layer. $\times 80$.
- 8 Small anchoring villus showing stroma penetrating compacta. $\times 80$.
- 9 Root of large villus showing masses of cells between stroma and syncytium.
 $\times 80$.

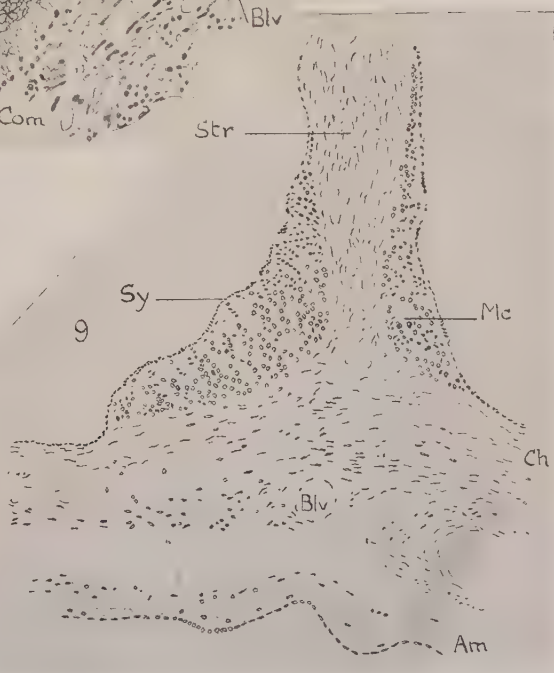
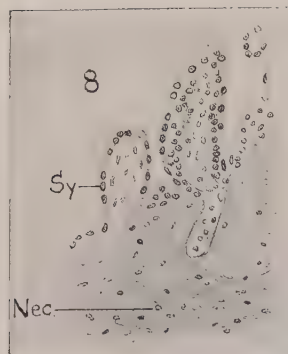
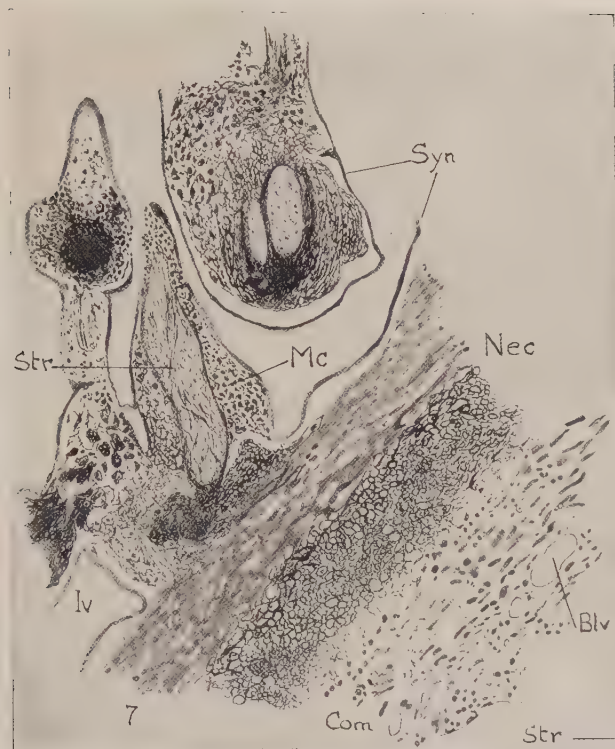
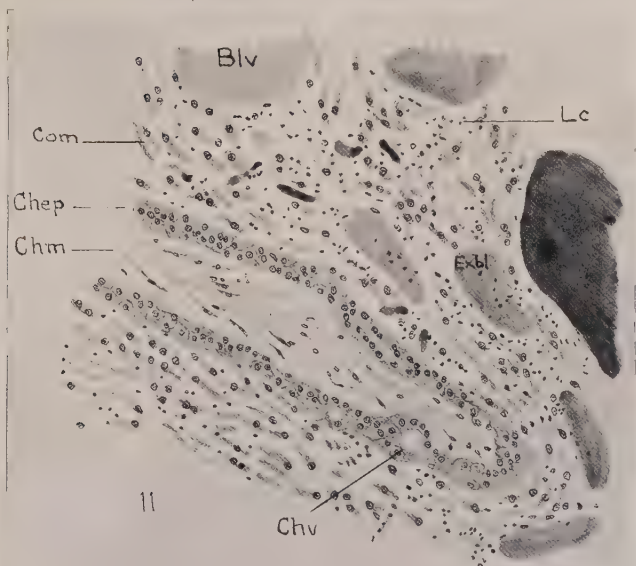


PLATE 4

EXPLANATION OF FIGURES

- 10 Edge of placenta to show 'closing plate.' $\times 12$.
11 Wedge of chorion cutting into compacta at point *X* in figure 10. $\times 113$.



DIFFERENCES IN THE TIME OF DEVELOPMENT OF CENTERS OF OSSIFICATION IN THE MALE AND FEMALE SKELETON¹

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THREE PLATES (SIX FIGURES)

"The bones of the female ossify in advance of the male. This is measured at first by days, then months, then years."²

The above observation was made after the study of 554 roentgenograms of the hands of children from three months to fourteen years of age. Two hundred of these were under seven years of age. This conclusion was based on the time of development of centers of ossification in the bones of the carpus; in the epiphyses of the metacarpals, phalanges, the distal extremity of the radius and ulna and the time of union of the epiphyses with the shaft in older cases.

Comparisons were made between brother and sister; twins of the same sex; twins of the opposite sex and the average of male and female hands of the same age. With these findings in view and the conclusions accurate, the first centers of ossification appearing in the embryo would be in the female.

Mall states the first centers of ossification develop in the clavicle at the sixth week. He did not make a distinction between the male and female. It is probable these early centers were in the female.

The conclusions in this paper are based upon the study of roentgenograms of 140 fetuses from ten and one-half weeks to thirty-eight weeks of age, of which seventy-one were male and sixty-nine were female; and one hundred new born babes from

¹ Paper read at meeting of American Association of Anatomists, December, 1921.

² Pryor. Bulletin State College of Kentucky, June, 1905.

a few hours to ten days of age, of which forty-eight were male and fifty-two were female.

Very few of the fetuses were under twelve weeks and the early centers could not be compared. The principal points to be compared were the centers of ossification of the calcaneus, the talus, the cuboid in the tarsus, the epiphyses of the distal extremity of the femur, the central extremity of the tibia and centers of ossification of the capitatum and hamatum of the carpus.

Centers of ossification have appeared in a large number of the bones of the human skeleton before differentiations of sex can be determined by the external genitalia. Felix³ states: "Sexual differences appear with the formations of the genital cord." "The differentiation of the reproductive gland into testis or ovary is actually to be observed." "This differentiation consists in the character of the male gland being developed in embryos of 13 mm. greatest length."

"By employing all these means the beginning of sexual differentiation may be determined in female embryos at a stage of 18 to 20 mm. in length."

It may be possible in the study of embryos to differentiate sex and make comparisons of the time appearance of centers of ossification several weeks before the external genitalia are characteristic.

In 32 mm. embryos the external genitalia are still in the indifferent stage and the estimated age is 9 weeks. At this time a large number of ossific points have appeared. Unfortunately with all cleared embryos that I have seen and studied the sex was not noted. I have therefore based my conclusions on the comparisons of embryos the 10th and 11th week; and fetuses the 18th week and older; and the new born from a few hours to 10 days of age.

³ Felix, Keibel and Mall Embryology, 1910.

TABLE 1

NO. ¹	MALE	AGE	NO.	FEMALE	AGE
	C. R. LENGTH			C. R. LENGTH	
884	56 mm.	10-1/2 weeks	1495	52 mm.	10-1/2 weeks
1556	57	" "	1242	56 "	" "
1234	58	" "	1548	57	" "
1346	59	10-5/7	1495	63	11
1471	62	11	1661	63	11
1462	64.5	11-2/7	935	66	11-1/2
1603	66	11-1/2	1578	67.2	11-5/7
1474	70.5	12	1661a	68	12
1900-29+	130	16	1478	70.5	12
1901-52	132	16	1242a	74	12-2/7
1902-81	132	16	1066	127.6	15
1510	142	17	1900-36	135	16
821	148	17	1900-28	139	16
946	149	17	1901-5a	139	16
1702	150	17	1901-47	141	17
521	151	17	1901-58	145	17
1901-56	152	17	951	148	17
1708	154	17	1068	148.6	17
1825	154	17	2282	143	17-3/7
747	155	18	2064	149	18
1103	155.6	18	1900-25	154	17-5/7
1901-54	160	18	1901-47	155	18
1901-60	162	18	1900-17	157	18
2034	165	19	1072	157.7	18
1383	165	19	1166	157.7	18
1901-53	168	19	1179	158	18
1902-82	170	20	1295+	159	18
1316	171	20	894	160	18
1900-16	172	20	1657	163	18
1480	172.5	20	2234	162	18
1185	173.5	20	1149	163.5	18-2/7
2257	170	20	745	165	19
1964	173.5	20	961	166	19
1924	177.5	20	2133	167.5	19-1/7
2211	177	20	2232	171	19-4/7
1096	179	21	854	175	20
1412	182	21	1691a	177.8	20
1469	183	21	1059	179	21
1900-11	183	21	817	185	21
2019	184	21	1195	181	21
1067	187	22	2279	183	21-6/7
1080	189.5	22	1143	188	22
2063	188.5	22	2220	190	22
1654	195	23	1306	189	22
2306	195	22-1/7	818	190	22
1901-55	195	23	1646	190	22

TABLE 1—*Continued*

MALE			FEMALE		
NO.	C. R. LENGTH	AGE	NO.	C. R. LENGTH	AGE
823	197	23	1900-2	190	22
2088	212	24	1900-1	194	22
2050	210	24	1489	198.4	23
2195	202	24	1498	199.4	23
1619	215	24	619	200	23
2249	216	24-2/7	1901-45	202	23
2192	219	24-1/2	2193	201.5	23-1/2
1900-27	218	25	666	205	24
2224	218	25	1764	205.3	24
2301	218	25-4/7	2011	208.5	24
1452	236	27	893	210	24
1651	236	27	1085	210	24
2167	211	25-4/7	1554	211.5	24
2223	226	26	1614	214	24
1792	240	27	2281	221	24-5/7
1503	250	28	2049	219	25
2089	239	27-5/7	1688	220	25
1396	262	29	2209	227	25
1800-30	264	29	2237	220.5	25-4/7
1691	270	30	1566	228	26
1508 ⁶	286	32	2184	226.5	26
1454	310	35	2239	323	37
1397	328	35	2238	317	37-2/7
2259	330	38			

¹ I am indebted to Dr. G. L. Streeter for the material in the above table.

There may be many differences to be noted in the early stages, such as the size of the centers of ossification in the vertebrae, the extent of ossification in the shaft of long bones, especially the metacarpals, metatarsals and phalanges.

Hess⁴ gives the result of the study of roentgenograms of 55 fetuses but does not note the difference between the male and female.

The ilium shows ossification in the female much in advance of the male. There is also a small center of ossification in the ischium in the female.

In the older fetuses the points to be noted are, the centers of ossification in the calcaneus, talus, cuboid, the distal epiphysis of the femur and the proximal epiphysis of the tibia.

⁴ Hess—American Journal of Diseases of Children, December, 1917.

TABLE 2

NO.	NAME	SEX	AGE	CAL.	TALUS	CUBOID	FEMUR	TIBIA
1	D	M.	3 days	7	5	0	3	0
2	C	M.	5 days	10	6	0	5	4
3	A	F.	6 days					
4	B	F.	2 hours	12	10	0	8	7
5	L	F.	8 mths. (Prem.)	8	4	0	2	0
6	H	F.	6 days	9	8	0	5	4
7	M	F.	6 hours	12	6	0	6	2
8	E	F.	7 days	10	7	2	5	0
* 9	S	F.	4 days	15	10	7	10	9
10	C	F.	4 days	10	7	0	7	5
11	C	F.	5 days	13	10	5	8	5
12	C	M.	3 days	8	5	0	5	4
13	D	F.	4 days	12	10	5	6	4
14	L	M.	5 days	13	10	0	7	4
15	K	M.	4 days	6	8	0	5	4
16	S	M.	1 day	12	7	2	4	2
17	D	F.	5 months	20	15	10	12	10
* 18	A	F.	4 days	13	10	5	9	7
19	M	M.	2 days	10	8	2	7	7
20	J	M.	5 days	10	7	0	6	5
21	M	M.	1 day	7	4	0	5	3
22	R	F.	1 day	14	10	5	8	5
23	C	M.	5 days	7	4	0	3	1
24	T	F.	8 days	12	8	4	7	4
25	R	F.	3 days	12	8	5	8	5
26	J	F.	3 days	13	8	1	8	5
27	M	M.	4 days	9	7	0	3	0
28	B	M.	10 hours	9	6	0	0	0
29	G	F.	5 days	8	6	0	3	0
30	M	M.	1 day	10	8	3	7	2
31	M	F.	25 days	10	8	0	2	0
32	C	M.	13 days	8	6	0	6	4
33	R	F.	4 days	8	7	0	8	5
34	twins	A	1 day	12	8	2	8	7
35								
36	S	M.	1 day	8	6	0	3	0
37	M	M.	1 day	8	6	0	4	0
38	H	M.	3 days	6	5	0	0	0
x39	M	F.	5 days	12	10	5	8	8
40	D	F.	3 days	12	10	3	8	4
x41	M	F.	5 days	10	8	5	8	6
42	P	M.	2 days	12	8	3	10	6
43	W	F.	7 days	12	10	4	10	8
x44	P	F.	7 days	14	10	4	10	8
45	R	F.	5 days	12	10	4	5	0
46	B	M.	4 hours	10	6	0	4	0
47	C	F.	3 days	12	10	0	9	7
48	C	M.	1 day	10	8	0	8	8

TABLE 2—Continued

NO.	NAME	SEX	AGE	CAL.	TALUS	CUBOID	FEMUR	TIBIA
49	H	F.	2 days	15	10	5	8	6
50	C	F.	7 days	12	10	0	8	6
51	C	F.	2 days	12	10	4	4	0
52	K	M.	5 days	10	8	0	6	8
53	W	F.	4 days	10	8	0	5	2
54	M	F.	5 days	12	10	5	7	7
55	K	M.	7 days	10	8	5	7	5
56	F	M.	4 days					
x57	N	F.	1 day	12	8	4	5	5
58	M	M.	6 days	8	6	0	5	0
59	D	F.	1 day	10	8	5	6	5
60	F	M.	3 days	10	10	0	7	0
61	} twins	C	8 mths. (Prem.)	8	5	0	0	0
62		M.						
63	S	F.	6 days	14	10	5	10	6
64	C	M.	6 days	10	8	0	4	1
65	H	F.	1 day	11	8	0	7	4
66	W	M.	3 days	8	8	0	7	0
67	P	M.	8 days	11	9	0	6	6
68	M	M.	2 days	8	7	0	3	0
69	S	M.	6 days	10	8	0	8	6
70	G	M.	7 days	9	7	2	6	5
71	Y	M.	1 day	7	5	0	5	3
72	D	F.	4 days	12	9	0	7	8
*73	H	M.	5 days	11	8	1	7	5
74	G	M.	2 days					
*75	G	M.	9 days	12	8	5	7	10
76	D	F.	3 days					
77	V	F.	4 days	12	10	1	7	8
78	H	F.	3 days	10	7	0	5	0
79	S	F.	4 days	12	10	0	7	4
80	M	M.	6 days	11	8	1	3	0
81	B	F.	7 days	13	10	5	8	8
82	B	M.	8 days	13	8	0	6	1
83	H	M.	4 days	8	6	0	5	3
*84	B	M.	2 days	10	8	2	5	5
85	W	M.	2 days	10	8	0	6	5
86	D	F.	(8 mths. Prem.)	8	6	2	5	1
			2 days					
87	G	F.	6 days	12	10	2	6	6
88	W	M.	4 days	10	8	0	4	1
89	C	F.	3 days	12	8	2	8	6
90	R	M.	1 day	10	8	0	5	6
91	Y	M.	1 day	6	5	0	4	2
92	J	M.	1 day	8	6	0	4	0
93	M	F.	6 days	11	6	0	6	7
*94	M	F.	1 day	10	8	3	7	4
95	S	F.	4 days	12	10	0	6	4
96	D	F.	4 days	14	9	5	4	4
97	S	F.	4 days	12	10	3	7	6
98	B	M.	4 hours	8	6	1	4	0
99	C	F.	6 days	10	8	5	6	4
100	D	F.	4 hours	10	8	1	5	0

In table 2 we have one hundred new born babes. I have given them in the order in which the roentgenograms were made, giving the centers of ossification in mm. of the calcaneus, talus, cuboid, the distal epiphysis of the femur and the proximal epiphysis of the tibia.

In a small group, centers of ossification have appeared in the capitulum and hamatum of the carpus.

These are the first instances in which centers of ossification have begun in the bones of the carpus before birth that I have seen and I believe that, in some of my roentgenograms of female hands, ossification of the capitulum and hamatum had appeared before birth. The evidence of this was the size of these centers. The best examples were a female of 6 months of age and one of 8 months of age.

It will be seen that the calcaneus has a centre of ossification in the female as early as the 18th week, showing evidence that it must have made its appearance as early as the 16th week of intra-uterine life. The size of this center as shown in table 2 would indicate that the calcaneus had begun ossification in all females by the end of the 6th month of intra-uterine life. In the male it may be seen as early as the 20th week but usually appears several weeks later.

The talus begins ossification several weeks later than the calcaneus and in every instance there is a large center of ossification at birth.

The cuboid shows a very irregular shaped center of ossification, sometimes scattered over an area of 2 or 3 mm. In some instances it would be impossible to say which was the beginning point.

Table 2 shows the cuboid present in all females but 16, that is, it is present in about 70 per cent; while in the male it was absent in 32 out of 48 instances. That is, it will be found present in only $33\frac{1}{2}$ per cent.

The presence of the distal epiphysis of the femur is thought by Bardeen⁵ and others to be evidence of maturity. He states, "This epiphysis has some medico-legal importance since its presence or absence has been utilized to determine whether a child is born at term."

⁵ Bardeen—Keibel and Mall Embryology, vol. 1.

In table 2 we see that the distal epiphysis of the femur is present in all instances but one, and that is a male.

It is certainly a positive sign of maturity.

TABLE 3

Most advanced in ossification

NO.	NAME	SEX	AGE	CAL.	TALUS	CUBOID	FEMUR	TIBIA
9	S	F.	4 days	15	10	7	10	9
11	C	F.	5 days	13	10	5	8	5
18	A	F.	4 days	13	10	5	9	7
22	R	F.	1 day	14	10	5	8	5
24	T	F.	8 days	12	8	4	7	4
25	R	F.	3 days	12	8	5	8	5
39	M	F.	5 days	12	10	5	8	8
40	D	F.	3 days	12	10	3	8	4
41	M	F.	5 days	10	8	5	8	6
43	F	F.	7 days	12	10	4	10	8
44	P	F.	7 days	14	10	4	10	8
49	H	F.	2 days	15	10	5	8	6
54	M	F.	5 days	12	10	5	7	7
57	N	F.	1 day	12	8	4	5	5
59	B	F.	1 day	10	9	5	6	5
63	S	F.	6 days	14	10	5	10	6
77	V	F.	4 days	12	10	1	7	8
81	B	F.	7 days	13	10	5	8	8
87	G	F.	6 days	12	10	2	6	6
89	C	F.	3 days	12	8	2	8	6
94	M	F.	1 day	10	8	3	7	4
96	B	F.	4 days	14	9	5	4	4
97	S	F.	4 days	12	10	3	7	6
99	C	F.	6 days	10	8	8	6	4
16	S	M.	1 day	12	7	2	4	2
19	M	M.	2 days	10	8	2	7	7
30	M	M.	1 day	10	8	3	7	2
42	P	M.	2 days	12	8	3	10	6
55	K	M.	7 days	10	8	5	7	5
70	G	M.	7 days	9	7	2	6	5
73	H	M.	5 days	11	8	1	7	5
75	G	M.	9 days	12	8	5	7	10
84	B	M.	2 days	10	8	2	5	5

The proximal epiphysis of the tibia is present in over 90 per cent of the females and in about 70 per cent of the males.

For the purpose of illustration I am selecting from table 3 the following Nos.: 9-18-39-41-44-57-94 from the female group and Nos., 73-75-84 from the male group.

Each of these show points of ossification in the capitatum and some in the hamatum.

In the female group the most advanced is that of No. 9, figure 3.

This girl is 4 days of age. The center of ossification in the capitatum is 2 mm. in diameter and that of the hamatum a

TABLE 4

Age one day and under

NO.	NAME	SEX	AGE	CAL.	TALUS	CUBOID	FEMUR	TIBIA
4	B	F.	2 hours	12	10	0	8	7
7	M	F.	6 hours	12	6	0	6	2
22	R	F.	1 day	14	10	5	8	5
34	A	F.	1 day	10	7	0	8	6
35	A	F.	1 day	12	8	2	8	7
57	N	F.	1 day	12	8	4	5	5
59	B	F.	1 day	10	8	5	6	5
65	H	F.	1 day	11	8	0	7	4
94	M	F.	1 day	10	8	3	7	4
16	S	M.	1 day	12	7	2	4	2
21	M	M.	1 day	7	4	0	5	3
20	B	M.	10 hours	9	6	0	0	0
30	M	M.	1 day	10	8	3	7	2
36	S-	M.	1 day	8	6	0	3	0
37	M	M.	1 day	8	6	0	4	0
46	B	M.	4 hours	10	6	0	4	0
48	C	M.	1 day	10	8	0	8	8
71	Y	M.	1 day	7	5	0	5	3
90	R	M.	1 day	10	8	0	5	6
91	J	M.	1 day	6	5	0	4	2
92	J	M.	1 day	8	6	0	4	0

shade smaller. Both of these centers show several weeks of growth. They evidently began ossification 2 weeks or more before birth. The roentgenogram of the lower extremities show a corresponding development. That of the calcaneus is 15 mm. the talus 10, the cuboid 7, the epiphysis of the femur 10 and that of the tibia 9.

The nearest approach to this in the male is no. 73, figure 4.

A. A male 2 days of age. In this hand there is a very minute point of ossification in the capitatum with a corresponding development of the centers in the lower extremities.

In no. 18, female 4 days of age, there is a small center of ossification in the capitatum with a corresponding growth of the other centers.

TABLE 5

Age from two to four days

NO.	NAME	SEX	AGE	CAL.	TALUS	CUBOID	FEMUR	TIBIA
9	S	F.	4 days	15	10	7	10	9
10	C	F.	4 days	10	7	0	7	5
13	D	F.	4 days	12	10	5	6	4
18	A	F.	4 days	13	10	5	9	7
25	R	F.	3 days	12	8	5	8	5
26	J	F.	3 days	13	8	1	8	5
33	R	F.	4 days	8	7	0	8	5
40	D	F.	3 days	12	10	3	8	4
47	C	F.	3 days	12	10	0	9	7
49	H	F.	2 days	15	10	5	8	6
51	C	F.	2 days	12	10	4	4	0
50	W	F.	4 days	10	8	0	5	2
72	B	F.	4 days	12	9	0	7	8
77	B	F.	4 days	12	10	1	7	8
78	H	F.	3 days	10	7	0	5	0
79	S	F.	4 days	12	10	0	7	4
89	C	F.	3 days	12	8	2	8	6
95	S	F.	4 days	12	10	0	6	4
96	D	F.	4 days	14	9	5	4	4
97	S	F.	4 days	12	10	3	7	6
1	D	M.	3 days	7	5	0	3	0
12	C	M.	3 days	8	5	0	5	4
15	K	M.	4 days	6	8	0	5	4
27	M	M.	4 days	9	7	0	3	0
38	H	M.	3 days	6	5	0	0	0
42	P	M.	2 days	12	8	3	10	6
60	F	M.	3 days	10	10	0	7	0
66	M	M.	3 days	8	8	0	7	0
68	M	M.	2 days	8	7	0	3	0
83	H	M.	4 days	8	6	0	5	3
84	B	M.	2 days	10	8	2	5	5
85	W	M.	2 days	10	8	0	6	5
88	W	M.	4 days	10	8	0	4	1

No. 84. B. Male 2 days of age. In this hand there is a small point of ossification in the capitatum and a corresponding development of the other centers.

In the hands of 52 females we find centers of ossification have developed in the capitatum or hamatum in 7 or nearly 8 per cent.

In 48 of the male hands we find 3 showing minute centers of ossification in the capitatum or nearly 3 per cent soon after birth but none before birth. Of course there is variation. The

TABLE 6
Age five days and over

NO.	NAME	SEX	AGE	CAL.	TALUS	CUBOID	FEMUR	TIBIA
6	H	F.	6 days	9	8	0	5	4
8	E	F.	7 days	10	7	2	5	0
11	C	F.	5 days	13	10	5	8	5
24	T	F.	8 days	12	8	4	7	4
29	G	F.	5 days	8	6	0	3	0
39	M	F.	5 days	12	10	5	8	8
41	W	F.	5 days	10	8	5	8	6
43	W	F.	7 days	12	10	4	10	8
44	P	F.	7 days	14	10	4	10	8
45	R	F.	5 days	12	10	4	5	0
50	C	F.	7 days	12	10	0	8	6
54	M	F.	5 days	12	10	5	7	7
63	S	F.	6 days	14	10	5	10	6
81	B	F.	7 days	13	10	5	8	8
87	G	F.	6 days	12	10	2	6	6
93	W	F.	6 days	11	6	0	6	7
99	C	F.	6 days	10	8	5	6	4
2	C	M.	5 days	10	6	0	5	4
14	L	M.	5 days	13	10	0	7	4
20	J	M.	5 days	10	7	0	6	5
23	C	M.	5 days	7	4	0	3	1
32	C	M.	13 days	8	6	0	6	4
52	K	M.	5 days	10	8	0	6	8
55	K	M.	7 days	10	8	5	7	5
58	M	M.	6 days	8	6	0	5	0
64	C	M.	6 days	10	8	0	4	1
67	P	M.	8 days	11	9	0	6	6
69	S	M.	6 days	10	8	0	8	6
70	G	M.	7 days	9	7	2	6	5
73	H	M.	5 days	11	8	1	7	5
75	G	M.	9 days	12	8	5	7	10
80	M	M.	6 days	11	8	1	3	0
82	B	M.	8 days	13	8	0	6	1

most advanced male shows greater ossification than the most retarded female. I have some evidence that an early or a retarded normal ossification is hereditary.

SUMMARY

Calcaneus	{ Female 18th to 20th week.
	{ Male 22d to 29th week.
Talus	{ Female 22d to 29th week.
	{ Male 24th to 32d week.
Cuboid	{ Female 30th week to shortly after birth.
	{ Male 38th week to several weeks after birth.
Femur	{ Female 25th to 30th week.
	{ Male 30th to 40th week.
Tibia	{ Female 28th to 40th week.
	{ Male 35th week to shortly after birth.

CONCLUSIONS

In the ossification of the human skeleton the female is in advance of the male from the earliest developments of centers of ossification in the embryo and throughout intra-uterine life, progressing from days to weeks and months.

After birth the differences are progressive up to the union of epiphyses with the shaft of the long bones which takes place with the female from 3 to 4 years in advance of the male.

PLATE 1

EXPLANATION OF FIGURES

- 1 Embryo of 52 mm. female. a. Roentgenogram, b. drawing.
- 2 Embryo of 62 mm. male. a. Roentgenogram, b. drawing.



PLATE 2

EXPLANATION OF FIGURES

- 3a Hand of female 4 days, No. 9. Roentgenogram.
- 3b Lower extremities of female 4 days, No. 9. Roentgenogram.

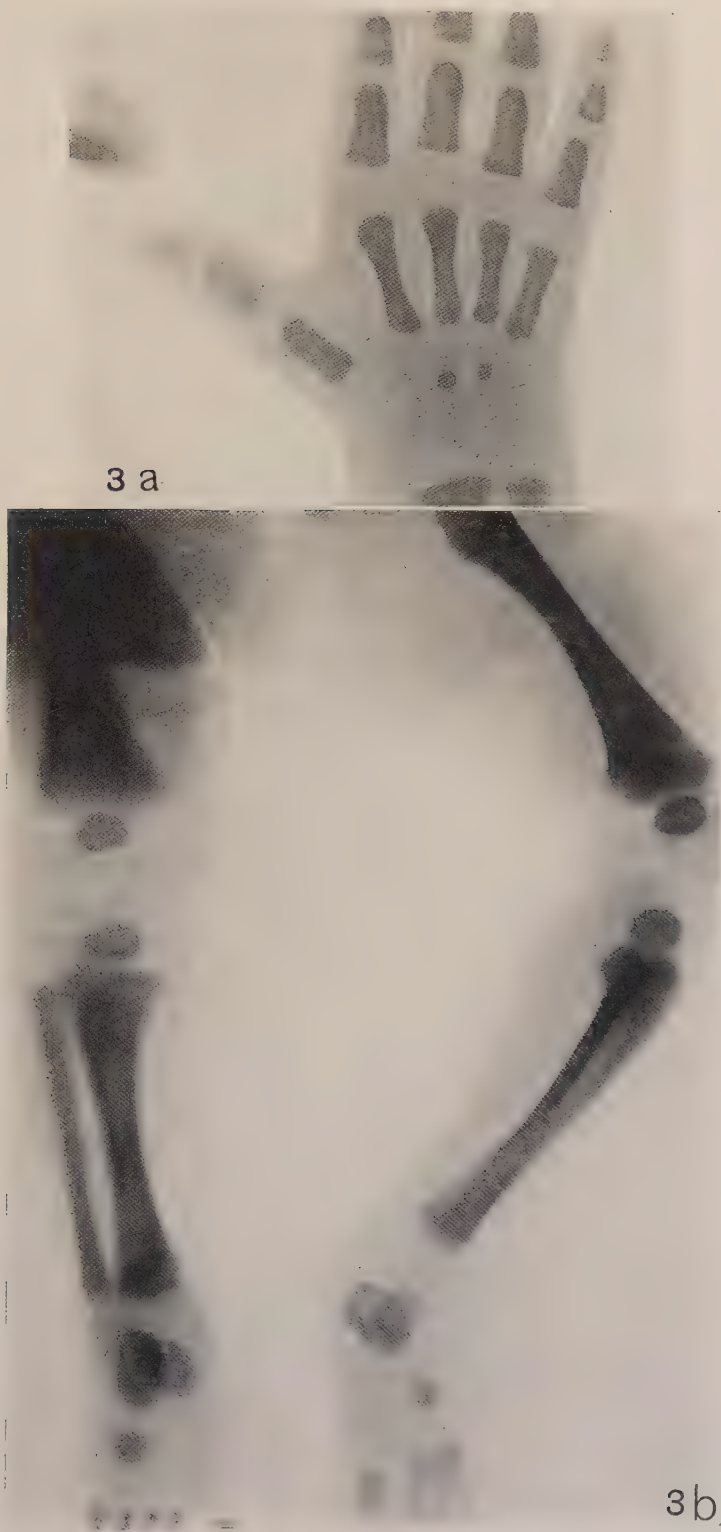


PLATE 3

EXPLANATION OF FIGURES

- 4a Hand of male 2 days, No. 73. Roentgenogram.
- 4b Lower extremities of male 2 days, No. 73. Roentgenogram..



THE ANATOMY OF THE SEMINAL VESICLES OF THE GUINEA-PIG, *CAVIA COBAYA*

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The earliest article which I have been able to find upon the subject of the seminal vesicles of the Guinea-pig is that of M. Andral, Fils, 1821. Andral concluded on the basis of electric stimulation that muscle must be contained in the walls of the vesicles but added that an anatomical examination failed to reveal it. When the date is considered this should not surprise us. Andral further stated that the seminal vesicles always are filled, and asked whether some of the liquid contents is the product of a secretory activity on their part. He apparently made no investigation on the question, but assumed that they store spermatozoa. The next work was that of Prevost and Dumas, 1824. These authors studied the male generative organs of many animals, and came to the conclusion that the spermatozoa (*animalcules spermatiques*) are the only constituents of the semen essential to fertilization. They noted the occasional presence of the 'white semen' from the ductus deferentes in the bases of the vesicles of the Guinea-pig, and stated that they occasionally found spermatozoa in that portion of the fluid which was in contact with the mucous membrane, even in the cul-de-sac. I, however, never found spermatozoa in the vesicles except at the base, but on examining the inner wall of the opened vesicle in water under the microscope, I found the innumerable minute bodies described below, which possibly may have been mistaken for spermatozoa at that time. Moreover, Prevost and Dumas stated that on many observations nothing was found in the vesicles except the true secretion, and that the function of the vesicles is evidently two-fold; i.e., to secrete a product of their own, and to serve as reservoirs for the other genital glands.

In 1884 Minot observed the large size of the seminal vesicles and the scarcity or absence of spermatozoa in them. This led him to make a brief anatomical study of the vesicles and to question the validity of the common belief that the chief, if not the only function of the seminal vesicles is to serve as a sperm reservoir. The work of Cannieu in 1892 has, unfortunately, not been available to me. M. de Pousargues ('93) made a study of the homologies of the so-called uterus masculinus and the orifices of the various accessory genital glands upon the verumontanum. He found a slit-like aperture on each side of the colliculus seminalis into which the eight ducts of the prostate on that side opened. In the median line on the colliculus he found a single depression, the vestibule of the uterus masculinus (vagina masculinus of other authors) with the seminal vesicles, the ductus deferentes and the uterus masculinus opening into it.

Max Rauther ('03) has done the most extensive work on the accessory genital structures of the Guinea-pig. He describes large, swollen, clear epithelial cells in the secreting gland, which might be called goblet cells. This author gave a very complete review of the literature but the fullest references on the anatomy and physiology of these structures are in Oppel ('04) and Marshall ('10). However, their sections on the Guinea-pig are taken largely from Rauther.

Everyone who has opened the abdomen of a male Guinea-pig must have been impressed by the large size of the seminal vesicles. Doctor Meyer, who kindly directed this study, first drew my attention to this matter by asking me how large human seminal vesicles would have to be, to be relatively as large as those of the Guinea-pig. The purpose of this paper is to present a brief study of the structure of the seminal vesicles of this animal, and to give an answer to that question.

On opening a Guinea-pig by a ventral mid-line incision, and reflecting the intestines, one comes upon two shiny, pearl-like blind, tubular structures, the seminal vesicles, which usually lie against the posterior abdominal wall, one in each paravertebral groove. However, since their mesenteries are only attached to the body wall near their base, after the manner of the human

appendix, the vesicles may be found anywhere in the lower abdomen. They have a diameter of a little less than 0.5 cm. and a length of 9 cm. and are not perfectly straight. Near the middle, each vesicle is usually thrown into a number of folds because it is longer than its mesentery. Small diverticula are occasionally found, usually at the free end. These are generally single and are rarely greater in length than the diameter of the vesicle at the point from which they spring. Diverticula are found most frequently in immature animals. Here they occur as a bifurcation of the tip. Their greater prevalence in the young would indicate that, as growth and increased sexual activity take place, they tend to lose their identity because of the expansion of the wall.

If traced toward their attached ends, the vesicles will be seen to emerge from the pelvis between the bladder and the ductus deferentes ventrally and the rectum dorsally. If the bladder and vesicles are raised, it will be found that the vesicles acquire their mesenteries posteriorly from the posterior wall of the pelvis at a point approximately on a level with the brim of the true pelvis. Caudal to this point for a distance of 1.5 cm., the vesicles and other pelvic organs are extraperitoneal.

The mesentery of each vesicle arises as a continuation of the peritoneal fold which contains the ureter, at a point where the later leaves the dorsal body wall at the brim of the pelvis minor to swing across the pelvic cavity to the bladder. It contains a fairly large artery, a branch of the hypogastric, which breaks up into three main branches supplying the bladder, the seminal vesicle, and the ductus deferens. According to Akutsu ('03) it also contains the hypogastric plexus, stimulation of which causes the contraction and emptying not only of the seminal vesicles, but also of the bladder and colon simultaneously.

Beneath the peritoneum, between the bladder and the bases of the vesicles, lie the proximal ends of the ductus deferentes. The fine areolar tissue which binds these structures together may be separated with comparative ease for a distance of 1 cm., but beyond this the organs become so intimately attached to one another that it is only with great care that the ducts and the

seminal vesicles may be freed to the point at which they are attached to the urethra, 1.5 cm. from the neck of the bladder. According to Rauther ('03) each ductus deferens joins its seminal vesicle a short distance proximal to the junction with the urethra, and a short ejaculatory duct is thereby formed. Branches of the tubular prostate surround the extraperitoneal bases of the vesicles on all sides except ventrally where, as just mentioned, they lie in apposition with the ductus deferentes. A prominent lobe of the prostate extends along the dorsal side of each vesicle for a distance of about 1 cm.

When, after being slit lengthwise, the vesicle is put into physiological saline solution and the inner surface exposed by washing out the contents, the wall is seen to have a soft, velvety appearance, of surprising thickness, for when looking at the vesicle intact, one gains the impression of extreme thinness. The wall is about 0.7 mm. thick. Upon close examination the fuzzy appearance is found to be due to the presence of innumerable minute transverse wave-like folds of the epithelium, resembling minute plicae circulares. Under the dissecting microscope these are seen to be distinct folds of the mucosa, having a length equal to twice the thickness of the rest of the vesicle wall, and separated from each other by a distance equalling their height. If dissecting needles are hooked into the epithelium and pulled in opposite directions, it is found impossible to stretch out these folds appreciably, thus showing that they are true organic structures and not due to folding of a redundant epithelium lying upon a contracted muscularis. However, a certain amount of folding undoubtedly does take place when the vesicles contract. With the exception of the proximal 5 mm., these folds characterize the appearance of the entire inner surface from base to tip. In the less mature pigs the folds are very much less abundant. They reach their greatest development in animals which have been most active sexually, but appear to a certain extent in the vesicles of very immature pigs also.

Upon examining the cross section of the vesicle with the low power of the microscope, the picture obtained depends upon whether the vesicle was in the normal resting condition,—i.e.,

full and turgid, when the animal was killed, or whether it had just been exhausted sexually. In the former case, the epithelial folds previously described are flattened out against the wall by the pressure of the contents, whereas in the loose, depleted vesicle, in which very little of the contents remains, the folds are very prominent projecting into the cavity of the vesicle so as nearly to fill it and suggesting the appearance of the middle portion of the human uterine tube. One is at first puzzled because the cross section of the vesicle shows the folds also in cross section, while in the freshly opened vesicle the folds are transverse. This seeming paradox is the result of the spiral or at least the oblique nature of the folds, which is so marked that a cross section of the vesicle very rarely cuts them longitudinally as would otherwise be the case. As is to be expected, the longitudinal section of the vesicle also shows the folds in cross section. The sectional view of the depleted vesicle shows that the folds very often are somewhat branching like the letter Y.

The epithelium is composed of a single layer of tall, nonciliated, columnar cells, resting on a fairly distinct basement membrane. The nuclei are coarsely, but the cytoplasm is more finely granular. Here and there between the bases of the cells, or in the basement membrane, are the smaller, transversely-disposed nuclei of the latter. They are much more elongated, and more deeply staining. As would be expected, the actively secreting cells of the recently depleted vesicle are large and edematous, and their nuclei are swollen, oval, and smooth in outline. In the turgid, resting vesicle, however, the cells are smaller, more cuboidal, and their nuclei are contracted, irregular, more deeply staining, and less oval in shape. No typical goblet cells were observed; nor was the presence of mucus, either in the cells or in the cavity of the vesicle, ever detected.

The epithelium rests on a submucosa which varies greatly in thickness. In the turgid vesicle it is flattened out and relatively inconspicuous, but in the released depleted condition it becomes more prominent. In general the submucosa has a thickness about equal to that of the epithelium, but in the folds it may constitute a considerable mass of loose areolar tissue, widely separating

the two epithelial surfaces of the fold, or as is the case in the turgid vesicle, it may be represented only by a small blood vessel and a very minute amount of connective tissue. In the actively secreting depleted vesicle, the submucosa is highly vascular.

Just external to the submucosa is a somewhat irregular layer of ordinary smooth muscle, the thickness of which varies with the turgidity of the vesicle and the distance from the base. In the turgid vesicle it has a thickness at the tip of about two and one-half times that of the epithelium, while in the middle portion it attains a thickness of ten or twelve times that of the epithelium. At the base it is somewhat thicker still. The fibers are disposed circularly throughout, with the exception of an irregular, longitudinal band with a width at its distal portion equal to one-sixth the circumference. This band is easily seen in the contracted vesicle directly under the attachment of the mesentery. At the tip of the vesicle it lies internal to the circular layer, but in the more proximal portions it is external to the circular fibers, and is more extensive. At the base, in some sections of contracted vesicles it may be traced completely around the periphery just external to the circular fibers. In the turgid vesicle in which the entire musculature is stretched, the longitudinal fibers become inconspicuous except near the base where a restricted band may generally be seen. The longitudinal fibers are not sharply separated from the circular and wherever the two come together there is a region where the fibers are oblique and irregular, and in places the one may be seen to be a continuation of the other in a new direction.

A layer of loose areolar tissue covered by indistinct, flattened cells surrounds the muscularis. At the point of the mesenteric attachment it is increased in thickness so as to equal that of the rest of the wall of the vesicle. Here a large artery and vein are to be found. In addition to these, smaller vessels and nerves are present as well as a good supply of lymph channels. Just external to the large vessels there is a fairly large, non-medullated nerve derived from the hypogastric plexus, which is situated in the root of the mesentery near the base of the vesicle.

If a vesicle is cut across at the base and the contents expressed, the latter is seen to be a slightly granular clear, thick fluid. Almost immediately upon exposure it coagulates in a manner suggesting the hardening of paraffin. However, the solidification is not complete for some time, the contents remaining in a moist mass, coarsely granular, more or less friable and while coherent not tenacious like paraffin or sticky like molasses. After exposure for an hour or more, the contents becomes surprisingly hard and firm.

If a small quantity of the fresh contents is placed in a drop of normal saline solution and teased out on a slide, the coarse granules become distinct. The whole contents appears to be entirely homogeneous, and the granules seem to be held together only by their own cohesive properties. If some of the contents is pressed out between a coverglass and slide, low magnification reveals the coarse granules to have an irregular appearance suggesting that of a berry, and to be made up of finer granules. When a vesicle is opened longitudinally and gently and immediately washed out with saline, the fluid, as well as the spaces between the folds of the epithelium, contains innumerable minute, spherical bodies, just perceptible to the naked eye. These resemble minute, opalescent oil globules. Among the smaller granules will be seen much larger ones of various sizes, but usually quite visible without a lens. These larger granules are irregular and berry-like in appearance. Their surface is studded with the small fine granules, and they are very obviously made up of the latter. This can be proved by pipetting off some of the fluid containing only the fine granules. After standing some time such fluid will be seen to contain large granules, provided there was a sufficient concentration of the fine granules in the solution.

The chemical nature of the contents of the seminal vesicles is obscure. Sufficient time for a thorough chemical analysis was not available, and that very interesting aspect of the subject was not investigated by me. Marshall ('10) and Oppel ('04) both give references to the literature on this subject. The contents is only slightly soluble in cold distilled water. Such a solution is not coagulated by 50 per cent acetic acid, but a cloudy ring is obtained by the careful addition of HNO_3 , and a marked

xanthoproteic acid reaction occurs when the substance is treated with nitric acid. Sudan III is not retained by a smear; nor does the solution reduce Fehling's, but the application of iodine stains certain parts a deep reddish brown. The reaction is neutral to litmus paper. A complete solvent for the substance was not found among the ordinary laboratory solvents.

No spermatozoa were found in the vesicle except in the proximal centimeter, where their presence seems to be a result of post-mortem diffusion, for the longer the vesicle remains in situ after the death of the animal, the farther the white, milky sperm invades it. No spermatozoa were found in any of the vesicles removed immediately after killing the pig.

Physically, two substances may be distinguished in a microscopical study of the stained sections: first, a homogeneous, colloid-like substance found in both the full and depleted vesicles; and second, a finely granular substance occurring only in the depleted vesicle.

The first substance is the hard, homogeneous, colloid-like material which forms the great bulk of the contents, and stains blue with Delafield's hematoxylin, and red with Van Gieson's stain. It is the only substance found in the full vesicle. In the sexually exhausted vesicle, a small quantity still remains in the center of the tube. This is no longer a structureless mass as it was in the resting vesicle, but is broken up into the irregular, berry-like masses described above. In the vesicles which had not been emptied sexually for several days before the animal was killed, this substance was absolutely without form or structure, but in animals which had copulated within a day or two of their death, the berry-like masses could be distinguished in the homogeneous ground substance which fills the vesicle, and which stains identically with the masses. The longer the time since the vesicles were emptied, the less distinct the masses become.

The second, or finely granular substance, was found only in vesicles which had been sexually depleted a short time before death. While containing much free space, such vesicles were not entirely empty, but contained much finely granular material which stained purplish to blue with Delafield's hematoxylin and

eosin, and brownish to red with Van Gieson. It was more plentiful at the periphery of the lumen or in the secretion among the epithelial folds, where it stained lighter, although it also occurred in the center of the vesicle intermixed with some of the remaining berry-like masses described above. In favorable places it was seen adhering to the epithelial cells from which it undoubtedly came.

Whenever each is found alone, these two substances are quite distinct both in their physical appearance and in their staining properties. But where the two substances are closely associated, as in the center of the secreting vesicle, this difference becomes less apparent, so that it is impossible to distinguish between them at the line of contact. Thus it is seen that the finely granular substance and the homogeneous, functional product are one and the same, and that the physical difference observed is only another evidence of the strong surface tension which the contents exhibit by the myriad globule-formation when studied fresh in saline solution.

The exact nature and physiology of the contents is to a large extent obscure. If a female is examined within an hour or two after successful copulation, the vagina will be found to be completely plugged with a very firm, white, granular mass, which is identical in appearance with the coagulated seminal fluid. There can be little doubt that this plug, which Lataste ('83) calls a 'bouchon vaginal,' is made up largely of the product of the vesicles.

A series of 21 Guinea-pigs between the ages of 5 weeks and 2 years was used for the determination of the relative mass of the vesicles. Each pig was denied food for 24 hours before death, to allow time for the gastro-intestinal tract to empty itself, thus largely eliminating food as a source of error. It was found necessary to place the pigs in pens free from sawdust during this period, for in the absence of other food, Guinea-pigs readily eat sawdust. They were not deprived of water, however. The animals were killed with illuminating gas, immediately after being weighed on a torsion balance. Immediately after death, the pig was placed upon its back and its greatest length measured.

It was then opened, and a few general notes made as to the nutrition and the amount of food remaining in the stomach and intestines. The gross appearances of the seminal vesicles and other genital organs were noted, especially as to whether they were swollen and turgid due to sexual excitement prior to killing.

TABLE 1
Absolute and relative mass of the seminal vesicles (in grams)

NUMBER OF PIG	AGE IN MONTHS	WEIGHT OF PIG	WEIGHT OF VESICLE					
			Right		Left		Both	
			Absolute	Per cent	Absolute	Per cent	Absolute	Per cent
18 ¹	1.3	168.9	.1	.06	.1	.06	.2	.12
13	3.0	408.2	.7	.17	.9	.22	1.6	.39
20	2.7	437.2	.7	.16	.7	.16	1.4	.33
14	3.0	481.5	.8	.17	1.0	.21	1.8	.38
15	4.7	504.0	1.0	.20	1.3	.26	2.3	.46
16	4.7	552.7	1.1	.20	1.1	.20	2.2	.40
19	3.5	586.2	1.1	.19	1.1	.19	2.2	.37
12	8.3	626.0	2.0	.32	2.4	.38	4.4	.70
17	7.6	699.3	1.2	.17	1.2	.17	2.4	.34
11	7.3	701.7	1.3	.18	1.2	.17	2.5	.36
3	11.0	704.7	1.5	.21	1.5	.21	3.0	.43
10	6.8	710.7	1.0	.14	1.3	.18	2.3	.32
9	11.0	861.7	1.4	.16	1.1	.13	2.5	.29
7	11.0	916.0	1.9	.21	1.8	.20	3.7	.40
2	17.0	968.4	2.0	.20	1.9	.19	3.9	.43
1	20.0	987.0	2.0	.20	1.8	.18	3.8	.38
21	24.0	999.1	4.0	.40	4.0	.40	8.0	.80
5	11.0	1001.9	2.2	.22	2.1	.21	4.3	.43
Average	9.0	714.5	1.5	.19	1.5	.20	3.1	.41

¹ Sexually immature. Not included in the averages.

After this general inspection, the vesicles were carefully freed from the surrounding structures, ligated at their junction with the urethra, and cut. Each was weighed, measured for length and diameter at uniform points, namely at the greatest diameter (middle) and 1 cm. from the base. The volume was then computed by measuring the physiological saline solution displaced by the vesicle when immersed in a burette containing that fluid

at 20°C. The results of these measurements are recorded in the accompanying tables.

Although the series used is too small to give a close approximation of the actual, a few conclusions would seem to be warranted.

TABLE 2

Absolute and relative lengths of the seminal vesicles

NUMBER OF PIG	WEIGHT OF PIG	LENGTH IN CM.	LENGTH OF SEMINAL VESICLES (IN CM.)					
			Right		Left		Average	
			Absolute	Per cent	Absolute	Per cent	Absolute	Per cent
18	168.9	13.6	3.20	23.5	3.00	21.1	3.10	22.3
13	408.2	19.7	8.00	40.6	7.86	39.9	8.93	40.2
20	437.2	20.2	7.90	39.1	7.40	36.6	7.65	37.9
14	481.5	20.3	7.13	35.1	7.79	38.4	7.46	36.7
15	504.0	21.5	7.95	37.0	7.60	35.3	7.77	36.2
16	552.7	21.4	8.46	39.5	9.30	42.5	8.89	41.0
19	586.2	21.7	7.97	36.7	7.81	36.0	7.89	36.4
12	626.0	21.7	8.00	36.9	8.49	39.1	8.24	38.0
17	699.3	22.9	7.44	32.4	7.22	31.5	7.33	32.0
11	701.7	23.2	9.10	39.2	9.12	39.3	9.11	39.3
3	704.7	23.2	9.10	39.2	9.20	39.6	9.10	39.2
10	710.7	23.0	8.48	32.9	9.00	34.8	8.74	33.9
9	861.7	23.5	11.10	47.2	11.00	47.0	11.05	47.0
7	916.0	24.1	9.95	41.3	10.00	41.5	9.97	41.4
2	968.4	24.0	10.53	43.9	10.53	43.9	10.53	43.9
1	987.0	25.4	10.29	40.4	11.05	43.4	10.67	42.0
21	999.1	25.7	12.90	50.1	13.65	53.1	13.27	51.6
5	1001.9	25.5	11.38	44.6	11.35	44.5	11.37	44.6
6	1002.3	25.0	10.10	40.4	11.30	45.2	10.70	42.8
Average	714.5	22.9	9.21	39.8	9.43	46.0	9.37	40.2

¹ Sexually immature. Not included in the averages.

In table 1, it will be seen that the individual difference in the weights of the vesicles on the two sides is within the range of error, for the average shows the weight on the two sides to be identical. Each vesicle is approximately 0.2 per cent of the body weight, and both average 0.41 per cent of this weight. A fair correlation seems to be present between body weight of the vesicles, although the weight of the vesicles increases more

TABLE 3

Diameters and volume of the seminal vesicles

NUMBER OF FIG	WEIGHT OF FIG	DIAMETER IN CM.				VOLUME IN CC.		
		Base		Middle		Right	Left	Both
		Right	Left	Right	Left			
18 ¹	168.9	.18	.19	.10	.12	.04	.04	.08
13	408.2	.31	.31	.38	.38	.59	.65	1.24
20	437.2	.23	.34	.34	.40	.71	.68	1.39
14	481.5	.32	.31	.41	.43	.81	.90	1.71
15	504.0	.30	.40	.49	.40	.95	.99	1.94
16	552.7	.34	.32	.50	.41	1.25	1.24	2.47
19	586.2	.30	.30	.46	.42	1.05	1.06	2.11
12	626.0	.40	.34	.59	.51	1.87	2.17	4.04
17	699.3	.30	.30	.48	.50	.90	1.05	1.95
11	701.7	.31	.41	.51	.48	1.70	1.53	3.23
3	704.7	.60	.62	.70	.61	1.74	1.81	3.55
10	710.7	.31	.30	.48	.50	1.33	1.41	2.74
8	837.3	.52	.52	.55	.62	2.66	2.48	5.14
9	861.7	.52	.38	.62	.62	2.77	2.56	5.33
7	916.0	.56	.43	.62	.58	2.27	1.95	4.22
2	968.4	.58	.49	.58	.61	2.07	1.93	4.00
1	987.0	.45	.35	.59	.56	1.90	1.74	3.64
21	999.1	.31	.31	.62	.61	3.65	3.65	7.30
5	1001.9	.30	.31	.56	.53	2.20	2.00	4.20
Average	714.5	.39	.37	.53	.51	1.69	1.65	3.34

¹ Sexually immature. Not included in the averages. Ruptured.

TABLE 4

Summary of average measurements on 18 Guinea-pigs

	WEIGHT	PER CENT OF BODY WEIGHT	LENGTH	PER CENT OF BODY LENGTH	DIAMETER		VOLUME IN CC.
					Base	Middle	
Right	1.5	.20	9.21	39.8	.39	.53	1.69
Left	1.5	.21	9.43	40.6	.37	.51	1.65
Both	3.1	.42					3.34
Average	1.5	.20	9.37	40.2	.38	.52	1.67

rapidly than that of the body during the period of active growth, as one might expect. However, pig no. 12 with a body weight of only 626 grams had vesicles weighing 4.4 grams, but no. 5 weighing 1001.2 grams had vesicles weighing only 4.3 grams. Since the former was 8.3 months and the later 11 months old, it is evident that the explanation for this difference does not lie in age. Upon referring to table 3 it will be seen that the combined volume of the vesicles from no. 5 nevertheless was slightly greater than the volume of those from no. 12. This is contradictory, to be sure, and undoubtedly is due to the difficulty in determining such small volumes accurately. An examination of the table also shows considerable fluctuation in the calibre of the vesicles in pigs of different weights; the smallest pigs often having vesicles of larger calibre, even when care is taken to exclude variations due to copulation.

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ORIGIN OF THE NOTOCHORD IN CHORDATES

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In a recent issue of *The Anatomical Record* Miss Harmon has described certain abnormalities of notochord and neural tube¹ in the chick, leading her to the conclusion that the former is of ectodermal origin.

Almost exactly identical conditions have been previously described by me,² including the paired condition of the notochord accompanied by paired diverticula of the neural tube in one embryo, and the continuity of the two structures in another. In the former of these two embryos the notochord further shows alternate expansions and constrictions, possibly corresponding to the condition displayed by it in later stages when it becomes constricted by the developing vertebrae, and leading me to the conclusion "that the same stimuli (probably in this case temperature changes) which serve to induce precocious changes of one sort (segmentation of notochord) may also serve to cause arrest of development in another direction (spina bifida)."³

Paired outgrowths of the neural tube may occur independently as shown in another embryo described in the same paper, although here the structures are not bilaterally symmetrical as they are in the former case.

In the second of the embryos described by me the continuity of neural tube and notochord is accompanied by a canal which places the cavities of each in communication with one another, and probably represents a neurenteric canal.

¹Harmon, Mary T., 1922. Concerning the origin of the notochord in the chick. *Anat. Rec.*, vol. 23, pp. 363-369.

²Young, Robert T., 1919. Some abnormalities of notocord and neural tube in the chick. *Quart. Jour. University of North Dakota*, vol. 9, pp. 231-234.

³*l.c.*, p. 232.

The question as to which germ layer gives rise to the notochord is, it seems to me, largely an academic one. In viewing the entire series of chordates from *Amphioxus* to the mammals, we find strictly homologous conditions throughout, although these conditions are greatly modified and to some extent obscured in the higher types in correspondence with the modified conditions of their development. In all we find the notochord arising from a sheet or cord of cells which is invaginated from the surface layer of the embryo at the anterior part of the blastopore or primitive streak. In *Amphioxus* we call this layer entoderm, for from it arises the gut tract. In the vertebrates the invaginated layer plays a progressively decreasing part in the formation of the gut, until in the amniotes it becomes entirely cut off from the latter except for the evanescent neurenteric canal.

Shall we then consider the notochord as ectoderm in amniotes, entoderm in *Amphioxus*, and neither "fish, flesh, nor good red herring" in the *Ichthyopsida*, when its ultimate source, its place of origin, and method of development are strictly similar in all? What we shall call it seems to me immaterial, provided we recognize its exact homology throughout the series.

That structures which normally develop in continuity with each other may abnormally become displaced and develop independently is also shown in the second of these embryos, for here some of the posterior somites, derivatives of gastral mesoblast, which normally arises in continuity with the notochord, appear to have developed independently thereof.

All of which goes to show the plasticity of living matter and the futility of attempting to apply exact definitions to its parts and processes.

THE SIGNIFICANCE OF THE BLOOD VESSELS WITH- IN THE ENAMEL ORGAN OF THE MOLAR TEETH OF THE ALBINO RAT

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SIX FIGURES

In a recent comparative study of the enamel organ of the developing incisor teeth of the albino rat and that of the kitten, I came to the conclusion that these enamel organs were at all times free of intrinsic capillaries. Subsequent to this investigation one of the blocks of rat's head was sectioned in my laboratory for class work. The sections showed clearly that in the case of the molar teeth of this particular 7-day albino rat the enamel organ contained numerous blood capillaries. As I was uncertain whether this unusual condition represented an individual or specific variation, other heads were sectioned in the region of the molar teeth. In every case the enamel organ was vascularized. Since my material was all from the same litter of rats, however, uncertainty persisted as to whether the condition in question was actually a specific character. At this point I turned all of my material over to Dr. C. C. Speidel, and suggested that he make a comparative study of the developing molar teeth of the albino rat and the common brown mouse.

The material employed by Doctor Speidel in his investigation included new-born mice, 15 to 21-day albino rat fetuses, and rats of the first week after birth. Doctor Speidel's study had progressed to the point where he was ready to describe an intrinsic blood supply as characteristic of the enamel organ of the molar teeth of mouse and albino rat when Addison and Appleton announced, through their abstract card of The Wistar Institute Bibliographic Service, similar results. Doctor Speidel's results are, therefore, confirmatory of the published conclusions of Addison and Appleton ('22).

The status of the question regarding the developing enamel organ as concerns a blood supply would seem at present to be as follows: In mammals generally the enamel organ of all teeth is non-vascular. Exceptions to this rule include Monotremes, Marsupials (see Addison and Appleton) and the molar teeth of rats and mice. Granting, as we must, that in certain forms certain teeth have vascularized enamel organs, the most important fact regarding this unusual condition, namely its significance, still remains undetermined. The belief that the correct answer to this question can be advanced at least a step further furnished the motive for this second contribution to the subject of the histogenesis of the enamel organ. The present results are based almost exclusively on conditions as seen in the enamel organ of the molar teeth of the 1-day-old albino rat.

Two quotations will indicate the fundamental points at issue.

Not until the blood-vessels have penetrated to the vicinity of the stratum intermedium is there a well-defined layer of enamel to be seen. There is thus possibly a connection between the presence of the blood-vessels and the initiation of amelogenesis (Addison and Appleton, p. 180). The tips of the capillaries may, especially in later stages, fuse at a certain point with the cells of the outer enamel epithelium, and so establish continuity between the capillary lumen and the intercellular spaces of the outer tunic. Red blood-corpuscles may thus infiltrate the outer tunic. They are later ingested by giant-cells formed by the fusion of cells of the outer enamel epithelium (Jordan, p. 397).

Granting a certain degree of inadequacy in separating a few sentences from their context in an effort to state succinctly a certain position, it seems nevertheless that these two quotations fairly indicate the two divergent interpretations of the presence of blood capillaries in the developing enamel organ. One view interprets them as accidental, without functional significance, as in the nature of a foreign body to be removed by phagocytic giant-cells. This view regards peripheral fusion and actual penetration as only different degrees of essentially the same process. The opposing view interprets the intrinsic capillaries as causally and functionally related to the beginning of amelogenesis, as a more efficient functional arrangement than a simple peridental vascular plexus, as a thing functionally advantageous.

Two questions closely related to the main issue concern the origin of certain phagocytic cells within the later enamel organ (Jordan, '21), and the presence of perivascular lymphatics in relation to blood-vessels within the stellate reticulum (Addison and Appleton, '22).

The salient facts concerning the enamel organ of the molar teeth of the new born to one-day old rat are: 1) The considerable width and wide-meshed character of the stellate reticulum over the coronal apex; 2) the great abundance of extra-vascular red blood-corpuscles at all levels of the reticulum; 3) the presence of a considerable capillary meshwork upon the surface of the enamel organ; 4) the presence of certain branching capillaries at all levels of the stellate reticulum, even immediately superjacent to the stratum intermedium; 5) the slightly swollen character of the endothelium of these capillaries; 6) the occurrence of wide pericapillary spaces, and, 7) the occurrence of occasional large mononuclear phagocytes engorged with disintegrating red blood-corpuscles.

A narrow layer of dentin occurs over the cusps, but only a mere trace of enamel is discernible and this only over the apices of the cusps. The beginning of enamel formation correlates rather with the beginning of erythrocytaphagic activity of the stellate reticulum than with the time of deepest penetration of the invading capillaries.

The abundance of blood vessels over the surface of the enamel organ can reasonably be interpreted only as a source of supply of the nutritive materials upon which tooth development, involving primarily amelogenesis, is dependent. The fact that in many teeth this peridental capillary net never penetrates the stellate reticulum would seem to indicate that actual penetration is not a functional necessity; in other words, that the stellate reticulum can transport from the peripheral blood supply to the basal ameloblasts the blood-vascular materials which may be necessary for amelogenesis.

What then is the significance of the occasional intrinsic capillaries of the stellate reticulum? The answer to this involves that of the significance of the presence of extravascular blood-corpus-

cles in the meshes of the reticular syncytium. Their significance is suggested by the fact that these corpuscles are in part phagocytosed by certain large round cells. This phagocytosis would seem to indicate that the red corpuscles are of the nature of foreign material. It does not even permit of the far-fetched opinion that the disintegrating erythroplastids may themselves furnish pabulum for the support of amelogenesis.

First, then, with regard to the intrinsic capillaries: Certain peripheral capillaries fuse with the outer enamel epithelium, thus establishing continuity between the capillary lumen and the 'intercellular' spaces of the reticular syncytium. This accounts for the presence of the superficial free erythroplastids of the stellate reticulum. Certain other capillaries, instead of fusing peripherally with the enamel organ, penetrate to variable depths within the stellate reticulum where the same process of intimate fusion between endothetium and reticular cells occurs (fig. 1). Thus again free erythroplastids find opportunity for passage into the meshes of the stellate reticulum (fig. 4).

These intrinsic capillaries are terminal; they are not part of a closed system. They end within the stellate reticulum, after a variable course which occasionally involves a partial return to upper layers of the stellate reticulum, by fusion with the reticular syncytium (figs. 2 and 3). This penetration by capillaries may be a general characteristic of molar teeth. Even in the molar teeth of the kitten (7-day old) a few capillaries can be found within the outer half of the stellate reticulum. That the capillaries in the rat's molar are actively invading the stellate reticulum, instead of being passively incorporated, is demonstrated by the occurrence of an occasional endothelial cell in mitosis (fig. 3). The nature of the correlation between an intrinsic blood supply of the enamel organ and molar teeth need not here detain us beyond the attempt to show that it has no essential functional relationship to amelogenesis. It may be suggested in passing that this correlation results from a relatively more restricted dental sac, following a relatively greater size of the molar teeth.

The perivascular spaces of the stellate reticulum do not admit of interpretation as lymphatics. They are not lined with endothe-

lium (figs. 1, 3 and 5). Their wall is occasionally jagged, the included septa representing portions of broken reticular syncytium.



Fig. 1 Photomicrograph of area of stellate reticulum, including a capillary. The capillary is surrounded terminally by wide spaces, simulating perivascular lymphatics. The letter p has the same position in this figure as in the drawing, fig. 5, a portion of this area. The lower border of this figure adjoins the stratum intermedium. Magnification about 600 diameters.

They are clearly fixation artifacts. Their general occurrence in sections results from the relatively greater contractility of the relatively robust endothelial cells following the application of fixing fluids.

The most difficult point concerns the origin of the erythrocytrophages. The possibilities include: 1) Origin from one or several layers of the ectodermal enamel organ; outer enamel epithelium, stellate reticulum, or stratum intermedium; 2) origin from endothelium; 3) origin from minute amounts of mesenchyme accompanying the invading blood-vessels; 4) origin from lymphocytes which find their way into the meshes of the stellate reticulum in company with the extravascular red blood-corpuscles.

Observations on the multinucleated phagocytic giant-cells in the enamel organ of the lateral incisors of the week-old kitten led me to the conclusion of ectodermal origin from some portion of the enamel organ. In the case of the molar tooth of the rat, the evidence again forces the conclusion that the mononuclear phagocytes of this material are derived from the enamel organ itself. While an endothelial or mesenchymal or lymphocyte origin cannot be ignored, or need not even be denied as subsidiary modes of genesis, the chief method of derivation is from cells separated from the stellate reticulum.

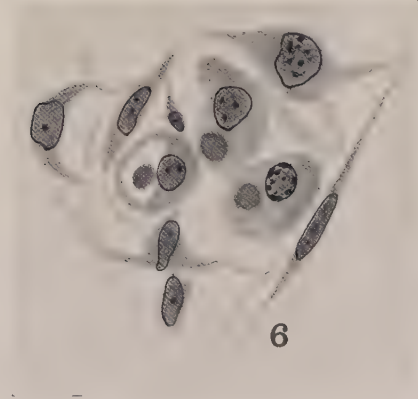
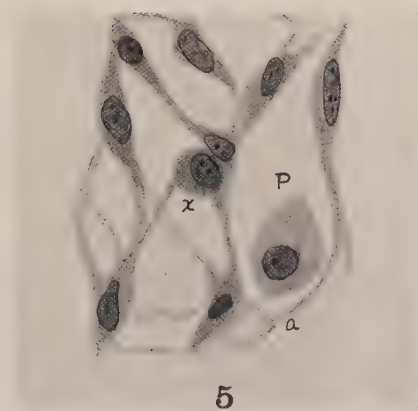
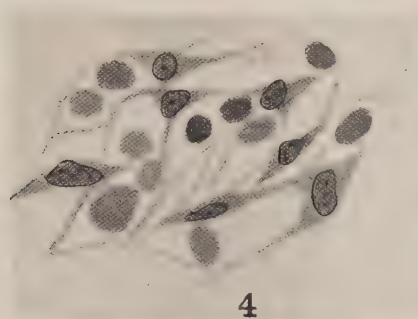
Fig. 2 Drawing of an area showing the continuity between the lumen of a capillary and the intercellular spaces of the stellate reticulum. At *a* the endothelium has fused with the stellate reticulum. At *b* is shown a red blood-corpuscle in a space continuous with the lumen of the capillary. This area adjoins the stratum intermedium. Magnification 1200 diameters.

Fig. 3 Area of stellate reticulum where it adjoins the stratum intermedium, showing the tip of an invading capillary fusing with the stellate reticulum (point marked *a*). At *c* is shown an endothelial cell in prophase of mitosis. The letter *p* is placed within a perivascular space, a fixation artifact.

Fig. 4 Area showing eleven red blood-corpuscles scattered throughout the spaces of the stellate reticulum. The larger body (below, at left) is a fusion product of several corpuscles. These corpuscles are shown as homogeneous spheroidal bodies. In the sections they vary in color from brownish red to a light straw color.

Fig. 5 Small portion of area shown in fig. 1. The letter *p* has approximately the same position in both figures. It is located in the perivascular space (fixation artifact) to the left of the capillary, and above a macrophage. At *x* is shown a macrophage in process of separation from the stellate reticulum. An area of fusion between endothelium and reticulum occurs at point *a*.

Fig. 6 Area of stellate reticulum showing three macrophages, each with an ingested red blood-corpuscle. The cytoplasm has a vacuolated structure. The red blood-corpuscle in one of the macrophages (at left) lies in a large cytoplasmic vacuole. The long fusiform cell at the right is an endothelial cell of the adjoining capillary. $\times 1200$.



These phagocytes have the appearance of large mononuclear leucocytes (figs. 5 and 6). Their nucleus, however, is always spherical, and about the size of that of a small lymphocyte. The nucleus in general appearance is practically identical with that of the cells of the stellate reticulum, except that in the latter case the shape of the nucleus is frequently oval or angular. This amounts to the same thing as saying that the nucleus of these phagocytes is practically identical with that of the endothelial cells of the invading capillaries; for the fusion between the endothelium and the reticular syncytium is so intimate, and the cells of the two are so closely similar, that it is impossible in some instances to tell where one ends and the other begins. However, some of these phagocytes may be seen to separate from the stellate reticulum (cell X, fig. 5) in areas free of capillaries. More generally these phagocytes originate adjacent to the capillary wall (cell P, fig. 5). These cells have a variable, but always considerable, envelope of acidophilic cytoplasm. They are generally of spherical form, but may be oval, more elongated, or even somewhat irregular. That the stellate reticulum is by no means an inactive or degenerate 'tissue' at this stage is indicated by the occurrence of occasional mitoses of nuclei of the syncytium.

An occasional endothelial cell of the intrinsic capillaries is seen to round up, and even, in some cases, to separate from the endothelial wall. As such they have an appearance similar to the phagocytes derived from the stellate reticulum. Some of these spheroidal endothelial cells remain attached and pass into mitotic division (cell C, fig. 3); others separate to mingle with the cellular content of the lumen. These endothelial leucocytes cannot in my material be further distinguished from the intravascular large mononuclear leucocytes. Both may eventually find their way into the stellate reticulum to function as erythrocytolytic macrophages.

I can find not the slightest evidence of an invasion of connective tissue at this stage in company with the ingrowing capillaries. Possible mesenchymal origin of intra-reticular phagocytes seems practically out of the question. The chief source of the erythrocytolytic phagocytes is the stellate reticulum. These cells

become engorged with erythroplastids in all stages of disintegration. In addition to the red blood-corpuscles within phagocytes (fig. 6), there are almost innumerable others lying free, either separately (fig. 4) or in groups, in the meshes of the stellate reticulum. Here they become swollen, gradually lose their hemoglobin, fuse into larger masses, and are apparently to some extent resorbed directly by the stellate reticulum without prior ingestion by separate ameboid cells. This process of extracellular resorption involves in some cases a stage of granular disintegration.

The evidence would seem to permit of no escape from the conclusion that the presence of capillaries in the enamel organ of certain forms, and of certain teeth, has no advantageous functional significance in relation to amelogenesis. Functionally, the condition appears to be rather a disadvantage than otherwise; for the red blood-corpuscles thus introduced into the stellate reticulum effect a response on the part of its cells which has the appearance of a reaction to foreign substances.

It must be emphasized, however, that this phagocytic response is relatively slight. This is perhaps to be expected in an ectodermal tissue. Only occasional pulpar cells separate from the reticulum to become erythrocytrophages. The vast majority of the extravascular pulpar erythroplastids are never ingested by such cells. They persist as swollen, to some extent fusing, sometimes fragmenting, globules. As such they become passively included in the plasmodial pulpar masses which arise at later stages in the stellate reticulum. These were previously described in the enamel organ of the incisor tooth of the kitten as multinucleated giant-cells (Jordan, '21). Besides erythroplastids, these incorporate, possibly also passively, certain enamel globules. These plasmodia in the kitten do not originate from invading connective tissue. They form part of the peridental cuticular membrane (of Nasmyth) and ultimately disintegrate, together with their content of red blood-corpuscles and enamel globules.

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A NOTE ON THE HEAD FORM OF FOUR HUNDRED
AND THIRTY-FIVE AMERICAN SOLDIERS, WITH
SPECIAL REFERENCE TO FLATTENING
IN THE OCCIPITAL REGION

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INTRODUCTION BY THE SENIOR AUTHOR

The effects of sleeping posture in early life upon the ultimate shape of the head and face may be greater than generally recognized. My four children have provided me with interesting confirmation of the plasticity of the head during the early years of life, with evidence that this may remain permanent later on.

The first girl slept on her back but moved her head a great deal from side to side with the result that her head became flattened behind but rounded and broadened in the temporal regions, and this has become permanent because today her head is broader and more rounded behind than it was at birth, or than that of the other children, or than that of either parent. Her face is also broader.

The second girl lay on her back when a baby and her head was kept in one position, on one side of the occipital region, and she obstinately persisted in keeping this position in spite of the efforts of both parents to make her lie in some other way, until finally we had to tie her head in different positions. This was not done until the head had become deformed. The forehead projected forward over her face and more on one side than another so that looked at from above and behind one cheek was visible but not the other. Today her forehead projects more than at birth, more than in the other children and more than in her parents.

The first boy slept as well one way as another and would stay in any position when placed there in comfort and his head seemed

to develop in a natural way. The second boy would lie on one side only until his head became unusually elongated and flattened on the side he lay on, and there was a rounded protuberance of the occipital region. This was partly corrected, but today his occipital region projects more than at birth, more than in the other children and more than in either parent.

In the Philippines the flattening of the occipital region was almost universal. This resulted in what some anthropologists have called a Malay characteristic, 'front Bombe.' The Filipinos sleep on hard surfaces with only a petaté or matting for a pillow, the back is the easiest position under the circumstances and it is natural for a normally rounded head to become flattened in the occipital region, straight up from the neck behind, and broadened and shortened. The cephalic index reaches extremely high proportions, sometimes almost 100, and in extreme cases the deformity is incongruous. The face seems to follow the head and becomes rounded and flattened.

Sometimes the head seems to have been pushed forward over the face without much flattening, sometimes the side is pushed out and sometimes the top of the head is pushed up with the flattening behind. Many shapes have resulted, but the most usual one is the flattening of the occipital region with broadening of the head and face.

With these and similar facts in mind when I examined the engineers of the University of Virginia Camp during the World War, I took particular care to look for any deformities that seemed to be the result of sleeping posture or that indicated any abnormal shape of the head in the way of occipital flattening. Any condition which seemed to warrant the term was called flattening, although it might be only some rounding and broadening of the occipital region. A greater proportion than usual of such deformities was encountered, but I am not sure that a more rigid examination with photographs would not show a larger percentage than that found. Other groups that are supposed to be normal may have as much or more of such deformations.

Thus the cephalic index which has been a fad and almost a fetich, and which has been losing its importance as a racial factor

of first importance by reason of many other studies becomes a factor of secondary consideration, although not to be relegated entirely to the background.

The following part of the paper is the work of the junior author with the advice and cooperation of the senior author.

The present study deals particularly with the head form in a number of individuals in which the back of the head was definitely flattened, and the correlation of this flattening with certain other features of the general shape of the head. Measurements of 435 American engineers were taken by the senior author during the World War at the University of Virginia Camp. In each case the following data were noted:

- Age
- Birthplace of subject
- Birthplace of subject's father and mother
- Birthplace of subject's grandparents
- Weight
- Stature
- Sitting height
- Head-length (antero-posterior diameter), head-breadth, head-height
- Face-breadth, face-height (menton-nasion), face-height (menton
crinion)
- Nose-length, nose-breadth
- Hair color
- Eye color
- Type

At the same time the general shape of the head was determined by palpation and those cases were especially noted in which the head was flattened behind. Of the 435 engineers, there were 38 whose heads were noticeably flat in the occipital region. A comparison of the head measurements of these 38 individuals with the head measurements of the other 397 revealed some differences.

Since flattening of the back of the head means a decrease in the head-length (antero-posterior diameter), it was thought that there might be a compensatory increase in the head-breadth and head-height measurements, and possibly also in the face-breadth. Accordingly the following indices were calculated for each individual: the head-breadth + head-height to head-length

index, the horizontal cephalic index, the bizygomatic index and the vertical cephalic index. The head-breadth + head-height to head-length index was obtained by multiplying the sum of the head-breadth and head-height in millimeters by 100 and dividing the product by the head-length in millimeters. The horizontal cephalic index, the bizygomatic index and the vertical cephalic index were obtained in the usual way. In the following table (table 1) may be seen a summary of these ratios for the 38 individuals with flattened heads, for 397 individuals with normal heads¹ and for the entire population of 435 individuals (the last group, of course, including both of the previous groups).

TABLE 1

	INDICES			
	Breadth+Height Length	Horizontal Cephalic	Bizygomatic	Vertical Cephalic
Head flat, 38	155.16	82.08	73.50	73.03
Head normal, 397	147.86	77.54	70.49	70.39
Total, 435	148.48	77.94	70.76	70.62
per cent increase in indices of flat	4.9	5.9	4.3	3.7

It will be seen from the foregoing table that in the individuals with the flattened head, the head-breadth + head-height to head-length index (155.16) is 4.9 per cent greater than it is in the normal individuals (147.86). This means that flattening of the back of the head results in an increase in the breadth of the head, or the height of the head, or both, in proportion to the length of the head. Comparison of the horizontal cephalic indices and the vertical cephalic indices shows that the greater part of this compensating increase is in the head-breadth. The horizontal cephalic index of the individuals with flat-heads (82.08) shows an increase of 5.9 per cent over that of the normal individuals (77.54), whereas the vertical cephalic index of the former (73.03) represents an increase of only 3.7 per cent over

¹ The term 'normal' is used to designate those individuals with no flattening of the back of the head.

that of the latter (70.39). Since the height of the head is measured from the external auditory opening to the bregma and since the bregma is rather far forward, it is clear that this measurement would not reveal the increase, or at least all of the increase in head-height that might occur farther back. The bizygomatic index in the individuals with flattened heads (73.50) shows an increase of 4.3 per cent over that of the normal (70.49). Evidently there is also a broadening of the face associated with the occipital flattening.

These four indices were then examined in each of the 38 individuals with flattened heads. The head-breadth + head-height to head-length index was slightly below normal in two cases, definitely below in one, and above in all of the other 35. In the one definitely below the normal average it is the head-height measurement which pulls it down. The head-height, as has already been suggested, is not so closely correlated with the flattening of the occipital region, and it is possible that some other factor, or factors, operated here to make the head-height relatively small (such as an early closure of the sutures at the bregma). The horizontal cephalic index is above normal in all cases except one where it is a very slight fraction below. Increase in this index is more closely correlated with flattening of the head behind than in the case with the other three indices. The bizygomatic index is markedly below the average of the normal individual in one case only, very slightly below in eight cases and above in all of the others. The vertical cephalic index is definitely below in three cases and somewhat below in four more. It is least correlated with the occipital flattening as compared with the other indices. It must be borne in mind that the vertical cephalic index does not take into account the back portion of the head (i.e., that portion behind the bregma), and that it is just that part, nearest to the occipital region, which would probably be most affected by the flattening. At the region of the bregma other factors might in some cases be more important in causing increase or decrease in head-height than occipital flattening, and this, indeed, is what the comparative figures suggest.

In four of the thirty-eight individuals with flat-heads the height of the head is the measurement which causes the large head-breadth + head-height to head-length index, while in three it is the factor causing a rather small head-breadth + head-height to head-length index. In eleven of the thirty-eight it is the breadth of the head measurement that very noticeably causes the large head-breadth + head-height to head-length index. In the other individuals both the head-breadth and head-height measurements operate almost equally to bring about the increased head-breadth + head-height to head-length index, the head-breadth measurement being slightly more important as a rule.

In general, also, the bizygomatic index increase over normal is fairly closely correlated with the horizontal cephalic index increase over normal. Widening of the back portion of the head (where the breadth of the head measurement is taken) as a result of flattening of the occipital region is transmitted also to the more forward portions, thus increasing the width of the face.

FLATTENING OF THE HEAD IN RELATION TO TYPES OF INDIVIDUALS

A study was next made of the relative effects of flattening of the back of the head in different types of individuals. The 38 individuals with flat-heads were grouped according to type and the four indices computed for each of the types. There were 18 individuals of the 'hyper-ontomorph' type (hyper) and 20 individuals of the 'meso-ontomorph' type (meso). The hyper-ontomorph type includes those descended chiefly from Mediterranean and Nordic stock. The meso-ontomorph type includes those derived from Kelto-Slav and Alpine stock. Many of the individuals of these classes are 'blends' or 'mosaics' of varying degrees, while some are of fairly pure racial type. In like manner, the normal population of 397 was divided into these two type groups, and the indices computed. There were 210 normal individuals of the hyper-ontomorph type, 172 of the meso-ontomorph type, and 15 with no type record taken. In table 2 these indices are given.

A comparison of the head-breadth + head-height to head-length index of the flat hyper (154.28) with that of the normal

hyper (147.38) shows that flattening results in a 4.7 per cent increase in this index. The horizontal cephalic index in the flat hyper (81.39) shows an increase of 5.5 per cent over that of the normal hyper (77.16); the bizygomatic index of the flat hyper (72.22) an increase of 3.3 per cent over that of the normal hyper; and the vertical cephalic index of the flat hyper (72.67) an increase of 3.7 per cent over that of the normal hyper (70.09).

TABLE 2
Flattening of the head within types

	INDICES			
	Breadth+Height Length	Horizontal Cephalic	Bizygomatic	Vertical Cephalic
18 Flat Hypers	154.28	81.39	72.22	72.67
20 Flat Mesos	155.90	82.70	74.65	73.36
38 Flat Total	155.16	82.08	73.50	73.03
210 Normal Hypers	147.38	77.16	69.95	70.09
172 Normal Mesos	148.51	78.00	71.15	70.76
15 No Type Record				
397 Normal Total	147.86	77.54	70.49	70.39
228 Total Hypers	147.92	77.50	70.13	70.29
192 Total Mesos	149.28	78.49	71.52	71.03
15 No Type Record				
435 Total	148.48	77.94	70.76	70.62

Per cent increase in indices of flat over normal within types

Flat Hypers over Normal Hypers	4.7	5.5	3.3	3.7
Flat Mesos over Nor- mal Mesos	5.0	6.0	4.9	3.7

In a similar way, a comparison of the indices of the flat meso and the normal meso shows that the head-breadth + head-height to head-length index in the former (155.90) is 5 per cent greater than it is in the latter (148.51). The horizontal cephalic index of the flat meso (82.70) is 6 per cent greater than that of the normal meso (78.00); the bizygomatic index of the flat meso (74.65) is 4.9 per cent greater than that of the normal meso (71.15); and the vertical cephalic index of the flat meso

(73.36) is 3.7 per cent greater than that of the normal meso (70.76). These percentage increases are shown in tabular form in the last part of table 2. It will be noted that there is a very marked type difference in the amount of increase shown by the bizygomatic index and no type difference shown by the vertical cephalic index increase.

It may be said, then, that flattening of the back of the head in individuals of the hyper-ontomorph type results in a somewhat smaller percentage increase in the head-breadth + head-height to head-length index and the horizontal cephalic index, and a markedly smaller percentage increase in the bizygomatic index than similar flattening of the back of the head in individuals of the meso-ontomorph type. In other words, flattening of the back of the head somewhat accentuates the characteristics of the originally broad-headed (meso) individuals relatively more than it tends to induce broad-headedness in individuals of the long-headed (hyper) type. The vertical cephalic index increase of the flat hyper over the normal hyper is the same as that of the flat meso over the normal meso.

DISCUSSION

These results show that flattening of the head in the occipital region is accompanied by and correlated with a definite increase in the breadth of the head and face and a somewhat smaller increase in the height of the head. It is clear, also, that in individuals of the meso-ontomorph type the increase in head-breadth, and face-breadth is relatively somewhat more pronounced than it is in individuals of the hyper-ontomorph type.

The question, of course, arises as to the cause of the flattening of the head and the significance of a relatively greater increase in the breadth of the face and head associated with this flattening in the case of the meso individuals. Of the various causes that might operate to bring about flattening of the occipital region the following may be mentioned. The flattening might be a hereditary characteristic, representing the somatic expression of the influence of certain germinal factors. In this case flat heads would run in families and be transmitted from one genera-

tion to the next. Secondly the deformation might be an entirely natural one occurring as a result of some developmental irregularity taking place during the embryonic period, or possibly in early infancy. Or, the deformation might be brought about intentionally by artificial means, by human agency. Various peoples have caused changes in the shape of the head of the young ones by the application of bandages, pads, supports, or other pressure appliances (i.e., the 'Flat-head Indians,' certain Pacific Islanders, etc.). In this manner may be induced various compensatory changes, pressure at one point being followed by bulging in other points. It is also possible for the deformation to be brought about by some special habit practiced during the early years of life. Head deformations caused by special sleeping postures are of this character. It is a fact that the heads of babies that lie in sleep in one position will be shaped and molded by the pressure on that part of the head which rests in the pillow. Lying on the side of the head would tend to increase the antero-posterior diameter and the height of the head and to decrease the breadth. Lying obliquely on the side and back of the head would tend to flatten that region and to cause protrusion on the part immediately in front or obliquely forward on the opposite side. And finally lying on the back of the head would tend to cause protrusion of the forehead and upper face, an increase in the head-breadth, face-breadth and head-height, and a decrease in the head-length. Pathological head deformations (caused by rickets, hydrocephalus, microcephaly, akromegaly, tumors, diffuse osteoporosis, leontiasis ossea) need not be considered here.

It seems entirely probable to the writers that the flat-heads of the 38 soldiers may have been brought about by sleeping posture habits, especially those of early life; by laying upon the back of the head. This pressure acting upon the soft and plastic head before the closure of the bony sutures would result in permanent flattening of the occipital region. Once a flattened surface is established this would tend to become the most comfortable and favorable spot for the head to rest on the pillow, and thus the flattening would tend to become further accentuated. The accompanying decrease in the head-length (antero-posterior

diameter) is compensated by an increase in the head-breadth, head-height, and face-breadth, as has already been pointed out.

The writers have no direct observations or data concerning the sleeping habits of these 38 soldiers during their early lifetime. Observations, however, by the senior author upon children of the Philippine Islanders confirm this interpretation.² The native child sleeps on a mat or directly upon the floor (the climate being too warm for a pillow) with the back of its naturally broad head supporting the weight of the head. As a result the head becomes very markedly flat behind, the breadth is increased and the entire cranium is pushed forward. This condition persists and is often regarded as a racial characteristic. Hrdlicka records pronounced occipital flattening occurring in the Navahos, Pueblos and other American Indian tribes as a result of sleeping on resistant head cushions (cradle boards)³. The senior author has also made observations in regard to the effect upon the general head form of sleeping upon the back of the head. Some of these have already been published in connection with another paper.⁴ These observations are in agreement with and confirm the interpretation given here.

SUMMARY

Of 435 American soldiers there were 38 with heads definitely flattened in the occipital region. A comparison of the head measurements of these 38 with the other normal 397 showed a marked increase in certain indices: an increase of 4.9 per cent in the head-breadth + head-height to head-length index; an increase of 5.9 per cent in the horizontal cephalic index; an increase of 4.3 per cent in the bizygomatic index; and an increase of 3.7 per cent in the vertical cephalic index. Flattening of the

² Bean, R. Bennett, 1909. III. Filipino types. Racial anatomy in Taytay. A. The Men. Philippine Journ. of Science, vol. 4, no. 5, pp. 401-409.

³ Hrdlicka, Ales, 1908. Physiological and medical observations among the Indians of southwestern United States and northern Mexico. Bur. Am. Ethnology, Bull. 34, pp. 79-81.

⁴ Bean, R. Bennett, 1909. III. Filipino types. Racial anatomy in Taytay. A. The men. Philippine Journ. of Science, vol. 4 no. 5, p. 405.

back of the head, therefore, results in compensatory increases in the breadth of the head, the breadth of the face and the height of the head. Flattening of the back of the head is most closely correlated with increase in the head-breadth, next most closely with increase in the face-breadth and least closely with increase in the head-height.

Division of the flat and normal individuals into meso-ontomorph type (Kelto-Slav and Alpine stock) and hyper-ontomorph type (Nordic and Mediterranean stock) and comparison of the effects of head flattening within each of these types shows that face-breadth and head-breadth are increased relatively more in the meso than in the hyper. Flattening, therefore, tends to accentuate in the meso the meso characteristics (broad head and broad face) more than it tends to modify the long head and face of the hyper in the direction of a broad face and head.

OBSERVATIONS ON THE OVA AND OVARIES OF THE GUINEA-PIG, *CAVIA COBAYA*

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THREE PLATES (EIGHT FIGURES)

GENERAL STATEMENT

This investigation suggested by Doctor Meyer was undertaken primarily to determine whether or not the presence of atypical ova could be detected in the ovary of the Guinea-pig by present cytological methods. However, as the examination of the ovaries progressed observations were also made on some other subjects. I wish to express my appreciation to Doctor Meyer for his many helpful suggestions and kindnesses during the preparation of this paper.

In all 24 ovaries from 19 different Guinea-pigs, ranging in age from two to four months were studied. Nine of these taken from seven animals were studied minutely and completely, every section being examined. Five other ovaries from four different animals were given a less complete examination.

Most of the ovaries were fixed in Bouin's, others in Zenker's solution. They were embedded in paraffin, sectioned serially, 5 μ thick, mounted and stained, some in Delafield's hematoxylin and eosin, others with iron haematoxylin and eosin.

ATYPICAL OVA

I was unable to find any report of an investigation upon atypical ova, but noted some indirect references to the existence of abnormal ova.

Jones ('97) described diseased ova, but she regarded disease as the causative agent in the production of very profound changes in the ova.

Loeb ('01) mentioned the fact that normal ova in Guinea-pigs may occasionally contain two nuclei, but he did not give his reason for judging them normal. It would seem that such ova should be termed atypical even if normal. Moreover, the condition must be comparatively rare, for I found no other mention of it nor did I observe it myself.

Lamb ('11) described hypertrophied ova in hens, which showed a very marked increase in the quantity of the yolk while the egg was still in the ovary. The eggs were described as being as large as the head of a new-born infant, oval in shape, yellow in color, laminated, and brittle, although they varied somewhat in structure.

Detlefsen ('14) in rather extensive studies on hybrids of wild and domestic Guinea-pigs, found the ovaries of two animals to be abnormally large, with greatly distended follicles. He said, "Abnormal ovaries of this type were not uncommon in other female hybrids which bore young and were otherwise perfectly normal in all respects. The viscera of many female hybrids were examined, but no data were taken on the occurrence of this type of abnormality." If these ovaries were really abnormal it is possible that they may have contained some atypical ova, but some of the ova were apparently normal enough to give rise to young.

Huber ('15) working on the albino rat, observed abnormal fertilized ova of a stage earlier than those showing implantation. The tubes and uteri were normal, and contained other ova that were normal. Huber expressed the tentative conclusion that these abnormal ova may result from abnormal germ cells, the abnormality of which may not show structurally. However, since these ova were fertilized this may mean an abnormality in either the sperm or ovum.

Long and Evans ('21) working on rats found that nine to ten ova liberated at ovulation are represented by only six to seven offspring carried to term, a failure of 30 per cent of the liberated ova to give rise to implantations. Davenport ('20) has made a similar observation, namely that in an examination of the number of embryos and corpora lutea in four sows, 36 per cent

of the ova liberated at the period of oestrous were not fertilized. But he considered this figure too high and stated that probably 10 to 25 per cent. of ova liberated "at favorable periods fail of fertilization." This however does not imply that the ova that failed of fertilization were abnormal. This is of course a very indirect and uncertain indication of the presence of abnormal ova for there is more than one factor responsible for the failure of so large a percentage of the liberated ova to develop.

The search for and detection of atypical ova is greatly interfered with by the phenomena of follicular atresia, and the degeneration of immature ova. As far as I have been able to learn there is no mammal in which continual degeneration of ova is not going on in the ovary, at least during the age of sexual activity. The stages which ova pass through during degeneration are numerous and varied; and granting that the abnormal ovum shows its abnormality structurally, the question remains as to how that difference may be distinguished from a stage in normal degeneration. Most of the advanced stages of degeneration are readily recognizable as such, but I do not believe that the earliest stages are as readily detected.

Loeb ('01) working on the Guinea-pig, and Asami ('20) working on the rabbit, both say that degeneration of the granulosa is the first sign of follicular atresia. Were this always the case, it would follow that an atypical appearance of an ovum within a follicle having the granulosa intact, is an indication of abnormality of the ovum. Unfortunately, however, I have seen follicles in which the granulosa was perfectly preserved although the chromatin of the nucleus of the ovum was massed and deeply stained, i.e., pycnotic. I do not believe that one is justified in saying that this nuclear pycnosis is indicative of an abnormal or atypical condition, because it is a well-recognized stage in degeneration and cell necrosis. It seems to me that the chances for such ova as these being liberated by ovulation, and being fertilized, are very slight indeed; for they are beginning to degenerate and in all probability they degenerate completely.

It is common to find the chromatin of ova greatly massed and stained deeply red, when there is no sign of degeneration in the

granulosa, and in cases where other ovular nuclei in the same section are stained normally. This change may very certainly be considered as a stage in degeneration.

Figure 1 shows a follicle from a pregnant Guinea-pig three and a half months old, presenting an unusual appearance. The follicular cavity is enclosed directly by cells which appear to be cuboidal epithelium. The ovum is partially surrounded by what appears to be a few granulosa cells. This follicle is located in the hilum of the ovary, a place not usually occupied by follicles, and is surrounded by numerous and fairly large blood vessels. The ovum itself is apparently degenerating as is indicated by the partial disintegration of its cytoplasm on one side, and by the appearance of its nucleus.

On following this follicle through the series it was found that in one place the cavity split into two parts, that is, there is a small diverticulum which rejoins the cavity. It is common to find tubules with an irregular course, and with numerous diverticula in this region of the ovary. These usually are the remains of the epoophoron, but this structure is a follicle for it contains an ovum. This interpretation agrees also with that of Walthard ('03) on the human ovary and of Heitz ('06) upon the ovaries of bovines. Both these investigators found islands of granulosa cells and also tubes of granulosa.

Figure 2 shows primary ovarian follicles lying in the superficial epithelium covering the ovary. One is seen to be covered by epithelial cells on its inner surface, although uncovered on a portion of its outer surface. The nuclei are decidedly pycnotic, which indicates that they may have already begun to degenerate. It seems to me very doubtful that these ova would ever migrate into the interior and mature. They could certainly much more conveniently move out. This same section also shows other primary follicles, normally situated, whose ova have very pycnotic nuclei. This indicates that degeneration may begin in very young ova.

The question of the place of origin of ova lying in the superficial epithelium is one that may be raised. They were found in the ovary of a Guinea-pig that was three months old, at which

age the ovary is normally mature. By this age one would ordinarily not expect ova to be forming still from the germinal epithelium. Hence these ova either formed in the external epithelium, or else, after originating within the ovary, have migrated out through the tunica albuginea. This latter explanation is improbable as the tunica is a very definite, and fairly thick layer, and then too, normally ova come to occupy a more central position within the ovary, probably by the ovarian stroma growing out around them. So it seems more probable that these ova formed in the superficial epithelium after the tunica albuginea had formed, and remained outside the latter. It is a question whether the two ova in figure 2 are degenerating, but it would appear that their ultimate fate will probably be either lysis in situ or liberation followed by lysis.

PHAGOCYTOSIS

In regard to the question as to whether or not phagocytosis enters into the process of the degeneration of the ovum various opinions are expressed. Several authors are guarded in their statements regarding it, and indicate that the steps in the process are not definitely known or understood. I have found no definite statement that phagocytosis of the ovum within the vesicular follicle does not occur.

Henneguy ('94) stated that in mammals granulosa cells and leucocytes do not enter the ovum until the last stages of regression, and that often the ovum atrophies without cellular elements taking part in the process. However, he says that in vertebrates other than mammals, degeneration is normally accompanied by the penetration into the ovum of a large number of migratory cells which act as phagocytes and destroy the cytoplasm of the ovum.

Stevens ('04) who did some work on the fate of the human ovum and Graafian follicle before puberty, described the invasion of the ovum by foreign cells, which he said, may not be phagocytes, but merely ingested granulosa cells. However, he felt quite certain that they are phagocytes. He said that they

may either be from the granulosa or leucocytes, but his opinion is that they are identical with granulosa cells, and that their ultimate fate is necrobiosis.

Kingery ('14) said that the fragments, resulting from degenerative fragmentation of the ovum of atretic follicles of the white mouse, are absorbed probably by the phagocytic action of follicular cells.

Charlton ('17) in a discussion of the fate of the unfertilized mouse egg, described the appearance of phagocytes between the cytoplasm and zona pellucida. He believed that from their appearance they are to be regarded as polymorphonuclear leucocytes.

Asami ('20) in working with rabbit ovaries, found that foreign cells entering the ovum do not phagocytise it as long as it remains healthy, but that when granulosa cells invade the ovum after it has undergone some degeneration, the invading cells remain healthy and act as phagocytes.

Sansom ('20) indicated that the interpretation of certain cells that he saw in the cytoplasm of degenerative fragments in the ova of the water vole, *Microtus amphibius*, is an open one. He says that they may be wandering phagocytic cells from the follicular epithelium, or cells that have formed inside the egg.

Loeb ('11), who did a great deal of work on the Guinea-pig, felt that his evidence indicated phagocytosis of the ovum by granulosa cells or by connective tissue cells of the theca. He said, "as soon as the interna becomes exposed, phagocytic cells (rounded-off interna cells) penetrate into the follicular cavity and these cells take up the debris of the granulosa." In a previous paper ('01) Loeb described progressive changes in young follicles, and stated that in the stage in which the follicular cavity becomes filled with connective tissue and then disappears, leaving the ovum surrounded by the theca interna, phagocytes may be seen eating up the remaining part of the egg. He further stated that in an earlier stage he had seen phagocytes destroying the egg even when no sign of atresia was present.

In a brief report, Evans ('16) presented evidence of the actual presence of macrophages in the ovary but it is not stated on what

animal this work was done. According to Evans, "True macrophages play an important rôle in follicular atresia and they are electively stained by vital dyes of this class (azo group). The later states of follicular atresia are consequently marked by brilliant deposits of the vital stain due to the intense dye granules in the macrophages concerned here. The macrophages must be considered as utilizing the products of the disintegrating ovum, towards which they are attracted and which they invade by an active penetration of the zona pellucida, these are the cells first seen in this act by Pfülgner and by Lindgren." Even if a cell which actively takes up particles of this dye may invariably be considered as being phagocytic, it may be significant that Evans did not seem to possess absolute proof that these macrophages actually ingest the ovum. Hence it is open to doubt whether the presence of these macrophages necessarily indicates or proves phagocytosis of the ovum by them.

In a later paper, Long and Evans ('22) state that corpus luteum cells do not stain vitally until they have attained a certain age, the young corpus cells not being penetrated by the dye, acting in this respect as they did when they constituted the granulosa cells of the ripe follicle. The illustration accompanying this point shows macrophages present which are loaded with the vital dye, but the remnants of the egg which are present and the young corpus cells contain no dye, and no macrophages have invaded the ovum.

In the ovaries of Guinea-pigs that I have examined I have seen no condition that I would take as evidence of phagocytosis of the ovum or of the granulosa. Figure 3 shows the remnants of a degenerated ovum in a follicle from which the granulosa has completely, and the theca very largely, disappeared. The ovum is reduced to the point where nothing remains but a structureless red-staining mass, with non-staining areas (vacuoles?). The ovum contains two cellular inclusions, one at a slightly lower level than the other. I consider both of these cellular masses to be degenerate because the nuclei are pycnotic, and the cytoplasm is rather coarsely granular, and stains somewhat irregularly. If one wishes to interpret these masses as phagocytic

in nature, the question may be asked, where did the contained granules come from? It is hardly fair to say that these two cells have already digested all the ovum, and are now degenerating in loco. I interpret the granules as being the result of the degeneration of the cytoplasm of the cells themselves, rather than ingested material.

A large number of ova in various states of degeneration were carefully examined. A few contained cellular inclusions, but in no case was a condition clearly suggesting phagocytosis observed. The degenerating ovum apparently may go through a number of different processes, e.g.: fatty degeneration, atrophy, chromatolysis, hyaline degeneration or fragmentation, or a combination of these five processes, as Henneguy ('94) pointed out. All these modes of degeneration have been observed, but it seems to me, that no matter what the earlier process of degeneration may have been the end result is lysis.

There is also the question of phagocytosis of the granulosa to be considered. Figure 4 shows a typical portion from a degenerating follicle. The granulosa cells farthest from the cavity are still normal, those most centrally located have completely degenerated, and those bordering on the edge of the normal cells are just commencing to show signs of degeneration. The degenerating granulosa from a number of atretic follicles was examined under high magnification and in no case was a phagocytic cell, as such, observed. Cells containing two nuclei were common. Cells containing dark staining fragments also were found, but in every case such cells appeared to be degenerate themselves, and the presence of two nuclei can readily be explained as being due to amitotic, or even of beginning mitotic division of a degenerating cell. As the cavity of the whole follicle is more or less strewn with fragments of degenerating cells there is no reason why such particles should not have found their way into the cytoplasm of preserved cells in the same way that carbon particles find their way from the peritoneal cavity into the lymphatics and blood capillaries in a living animal, as MacCallum ('03) found.

In order that a process be called phagocytosis it seems to me that there must be an active engulfing of extra-cellular material by a living cell. Hence until it is clearly demonstrated that such a process takes place within the ovary of the Guinea-pig, I do not see that we have any conclusive evidence of phagocytosis of the ovum in the disappearance of the granulosa or ova from atretic, or healthy, follicles.

DEGENERATIVE FRAGMENTATION

In the literature there is some confusion as to the use of terms, and some difference of opinion as to the interpretation of certain changes that the ova within the ovary may undergo.

Henneguy ('93) stated his views very clearly by saying "Mes observations sur la dégénérescence des ovules des mammifères confirment et étendent celles de Flemming et de Schottländer. Elles établissent que l'ovule des follicules en voie d'atrésie peut présenter, non seulement une maturité précoce, se traduisant par l'apparition d'un fuseau directeur et d'un globule polaire, mais aussi un commencement de segmentation irrégulière, parthénogénésique." A later paper (Henneguy, '94), in a review of the modes of regression of the ova, gives degeneration by fragmentation as one of the processes. He believes that it may be regarded as the last stage in chromotolysis of the ovum, and that in some cases it may lead to an irregular segmentation. In the latter case the chromatin is resolved into small irregular masses which are dispersed into the cytoplasm. Each mass of chromatin is said to act like a little nucleus and give rise to a rudimentary 'karyodiérétique' figure. Although the cytoplasm fragments into irregular masses Henneguy nevertheless considers it to be a beginning of parthenogenesis.

Van der Stricht ('01) also made observations on follicular atresia in the bat and concluded that the oöcyte of the second order forms a cleavage spindle and divides parthenogenetically. He traced what he interprets as normal cleavage to the six-cell stage.

Athias ('09), who made a very careful study of the divisions of the ova in atretic follicles of the dormouse (*Eliomys quercinus*), observed regular mitotic figures with spindles which were always bipolar. Ordinarily the chromosomes were very massed, but in some cases they could be counted. In one case he observed two spindles in the same ovum. He made the interesting, and it seems to me, very significant observation that, "Le cytoplasma des oöcytes qui présentent des figures de division caryocinétique possède, en général, une structure plus ou moins nettement réticulée, surtout dans les pièces fixées au Zenker. Il y a de fines granulations, souvent très nombreuses, qui cachent parfois le réticulum des vacuoles de dimensions variables et plus ou moins nombreuses. Dans les ovaires fixées au liquide de Flemming la structure granuleuse du cytoplasma ovulaire est plus accentuée; il se montre parsemé de boules graissuses, noircies par l'acide osmique." This statement certainly suggests that the ova containing spindles show degeneration in the structure of their cytoplasm and illustrations accompanying Athias' article bear out this fact.

In discussing whether or not fragmentation of the ovum is parthenogenesis, Athias quoted Bonnet as saying that one should not compare a fragmentation which gives rise to unequal-sized pieces, some nucleated and some not, to regular mitotic division. Athias observed that in the dormouse mitotic division is a product of maturation, throwing off the first polar body, and rarely the second. If the second polar body is given off it is generally in an atypical manner and is always followed by degeneration of the ovum. Athias is in accord with Sobotta and Bonnet in believing that the phenomena under consideration are regressive and characteristic of ova towards the end of the period of development and not evidences of parthenogenetic development.

Sansom ('20), in work on the water vole (*Microtus amphibius*), distinguished between degenerative fragmentation and parthenogenetic development. In the former condition the cytoplasm of the ovum was degenerate, the fragments were often multinucleated, and mitotic figures were absent. However, in parthenogenesis the eggs were seen to go to a four blastomere stage

and then degenerate. Sansom described the cleavage of ovarian eggs with the formation of spindles.

Kingery ('14) studied the ovaries of sexually immature white mice. In the atretic follicles he observed abnormal maturation spindles, which he considered not to be cleavage spindles. The ovum fragments and, "The process is one of degenerative fragmentation and not parthenogenetic cleavage."

Addison ('17), who observed the fate of the unfertilized egg in the white mouse, found that the egg often segmented, but in no case did he observe a cleavage spindle. Hence he concluded that the changes are similar to those of atresia, and are degenerative and only superficially resemble parthenogenesis.

American anatomists, who have perhaps done the most work on Guinea-pig ovaries, do not seem to be in accord on this question. Stockard and Papanicolaou ('17) studied a very large number of Guinea-pig ovaries in connection with changes during the oestrous cycle and correlated changes in the uterus and ovaries. They found that most of the atretic ova degenerate before the formation of a polar body, but some complete their maturation. In all the cases observed by them the ova of the atretic follicle degenerated and the nucleus broke up into irregular pieces soon after ovulation had taken place. These authors further stated, "We failed to find anything to indicate a tendency toward parthenogenetic divisions in the many specimens we have examined as Leo Loeb reported for these animals."

Loeb ('01), who also examined several hundred ovaries, carefully described what he termed progressive changes in the ova in some of the atretic follicles. He also described the formation of spindles with the liberation of the first polar body and the later formation of many small segments. In a later paper, Loeb ('11) described what he termed 'early spontaneous parthenogenetic embryos' in the ovaries of Guinea-pigs even to the formation of "a chorionic vesicle with trophoblast, and plasmodia and syncytia penetrating into the neighboring tissues. There is present also a structure which is probably to be interpreted as a neural tube." Proof that the structure described by Loeb is a developing embryo is based on his observation that aberrant blastomeres

can not be found in Guinea-pig ovaries; that the embryonic structures are present in 10 per cent. of all Guinea-pigs under six months of age; and that they are located in the cortex of the ovaries in follicle-like cavities. Loeb further stated that since the histories of some of the animals were known the possibility of intraovarian fertilization by spermatozoa may be excluded. In a still later paper Loeb ('15) described two more cases of embryonic structures in the Guinea-pig ovary. He says, "Our new observations render it therefore certain that a fargoing parthenogenetic development of the ova takes place in the ovaries of a relatively large number of Guinea-pigs, leading in the first place to the formation of placental structures, in some cases, however, also to the formation of embryos in the stage of the germ layers."

Newman ('13) reported the steps in what he terms parthenogenesis in the ova of atretic follicles in the armadillo ovary. He stated that in advanced follicular atresia there is an invasion "of stroma cells and leucocytes that feed upon the disintegrating egg material and form cell masses that have been interpreted by some authors as the products of embryonic development." He suggests that this may be the explanation of the structures Loeb termed 'placental tissues' in the Guinea-pig ovary.

Long and Evans ('22) state that the unfertilized ova of rats degenerate and undergo a degeneration consisting of "unequal, direct fragmentation, or segmentation, of the protoplasmic mass which may or may not carry with it unequal sized nuclei." In follicular atresia there may be "a rather orderly imitation of blastogenesis, or parthenogenesis, as L. Loeb has called it. The same thing may also occur in the case of an ovum retained in a corpus."

In the ovaries thus far examined no structure which could possibly be interpreted as parthenogenetically developing ova were observed. The fragmented ova corresponded with those described in the literature as undergoing 'degenerative fragmentation.' The granulosa of these follicles was always degenerate, the fragmentation of the ovum irregular, the cytoplasm deeply stained with eosin and usually vacuolated, and some

fragments contained several, and others no nuclei. The whole picture was one of degeneration,—a retrogressive process.

Kirkham also found that the first evidence of degeneration of the premature follicles in the mouse occurring between the seventeenth and the fortieth day is evidenced by "chromatolysis of the follicle cells which may or may not be followed by the maturation or fragmentation of the egg. In no instance has any normal maturation spindle or polar body been seen in this material." However, according to Heitz ('06), the first degenerative changes in atretic follicles in calves are seen in the nucleus and the nucleolus.

So far I observed eight spindles in ova. Seven were of the type shown in figure 5, a low power view of the follicle containing it is shown in figure 6. It is evident that this follicle is degenerating, as the disintegrated, dark-staining granulosa shows. The ovum also has a degenerate appearance and is somewhat vacuolated. The cell border is partially gone, and presents a crenated appearance. The spindle is perhaps concerned in the formation of the first polar body. The chromosomes are not distinct, but closely packed or fused together, and the rays of the spindle are somewhat irregular. This would indicate that the nuclear material of the ovum was also in a retrogressive condition. These facts it seems to me, indicate that the entire ovum although in mitosis is in a regressive, rather than in a progressive state. There is a possibility, to be sure, that the spindle was formed while the ovum and follicle were still in a normal or progressive state of development, and that degenerative changes overtook it. However, since the mitotic state is a fairly early one, and since the degenerative changes in the follicle are fairly far advanced, it seems to me entirely possible that the mitotic figure began to form in a follicle already on the down grade. We know that amitotic division may take place in normal proliferating tissue such as regenerating liver cells, and the division may also be mitotic. It is generally accepted that degenerating cells often undergo amitotic division, and why should they not just as well undergo mitotic division, at least partial if not complete?

The spindle in figure 7 is another of this same type. The granulosa of this follicle is very degenerate, especially in the portion not immediately surrounding the ovum. That the cytoplasm of the ovum is also degenerate is indicated by the irregularities in its staining and by the vacuoles. The spindle is apparently better preserved than the one of figure 5, the chromosomes being more distinct.

The spindle shown in figure 8 is at a more advanced stage than that previously described. But in this follicle also the granulosa has begun to degenerate. This degeneration is most marked in the portions of the follicle not shown in the photograph. The cytoplasm of the ovum is vacuolated and the chromosomes are massed together, indicating degeneration.

If a spindle were found in a non-degenerate ovum contained in a follicle with intact granulosa, and if there were evidence that the mitosis was not merely the maturation just prior to ovulation, it might be regarded as a progressive change, which if followed by regular division would be a step in parthenogenesis. But in the ovaries which I have examined I have found no evidence of the presence of a regular or mitotic segmentation in an ovum with normal-appearing cytoplasm, contained in a follicle with intact granulosa. Thus my findings are in accord with those of Athias, Bonnet, Kingery, Addison and Charlton: that is, that the apparent division of ova is actually a degenerative fragmentation, and that the spindles are probably of the first maturation although contained in degenerating cells. My observations also confirm those of Stockard and Papanicolaou in the absence of any evidence of a parthenogenetic development of embryos in Guinea-pig ovaries especially, as observed by Loeb.

CYSTS

The presence of cysts in the ovaries of Guinea-pigs is very common. In fact most of the ovaries examined contained numerous small cysts. There were clear cavities lined by low cuboidal epithelium, with many diverticula so that portions of ovarian stroma might extend into a cyst, and become completely

isolated. This suggests that as the cyst grows a portion of its wall may work its way through the stroma as a diverticulum which may again join the main cyst, completely surrounding ovarian stroma.

Other cysts whose nature is best described by the term cyst-adenoma were also observed. The lining cells were cuboidal and columnar in character, in some places simple and in others stratified. Masses of these cells also extended into the cavities of the cysts, being supported by a delicate framework of connective tissue.

THE EFFECT OF LIGATION OF OVARIAN VESSELS

In an attempt to gain information upon the effect of defective nutrition of the ovary on the degeneration of ova and follicles, a series of seven Guinea-pigs was operated on and the ovarian vessels ligated with silk. The Guinea-pigs were killed as follows: Two after one day, and one each after two, three, six, eight, and twelve days.

With one exception, which will be described below, the operative procedure and results were practically identical. In two of these six cases a single ligature was made just below the hilum of the ovary; in the other four cases two ligatures were used so as to divide the structures entering the hilum into two separate groups.

In each case the ovary at necropsy was much swollen and dark-red in color, looking like a hematoma. The exceptional case was in one of the animals which was killed one day after operation. In this case three ligatures had been used. Two were placed on the structures entering the hilum dividing them into two separate groups, and the third was placed just below the hilum and included all the structures. At necropsy the ovary appeared white, and normal, except for a slight opacity. There was no hematoma of the ovary, but there was a small swollen dark-red mass in the tissue just below it.

Since there is no apparent means by which blood could have entered the ovary after ligation, these results are rather difficult

to explain. At first thought one would conclude that the ovarian veins had been completely occluded, but that some artery or arteries had entirely or partially escaped ligation. But it is difficult to see how this could have been the case, for the ligatures entirely surrounded the structures enveloping the ovary, and were drawn very tight.

Four of these ovaries were sectioned serially and studied microscopically, a description of each follows:

Ovary no. 49—Vessels ligated for one day. Grossly the ovary was swollen and dark red in color. Microscopically the most striking thing was the extensive degeneration of the ovarian tissue. The entire central portion of the ovary stained a brick red with eosin and contained a few blue stained degenerate nuclei presenting the typical picture of coagulation necrosis. Adjoining this central necrotic area was a region containing many greatly distended and packed blood vessels. There was also considerable hemorrhage as indicated by the masses of blood cells in the tissue. This hemorrhagic region lay beneath the tunica albuginea, which latter structure together with the surface epithelium showed little if any change. Some of the peripherally-situated vesicular follicles gave indication of their former structure, but all were quite far degenerated. The entire picture was of an acute passive hyperemia, or a venous stasis, with hemorrhage. The degeneration of the follicles was from without inward.

Ovary no. 51—Vessels ligated for one day. The gross appearance of this ovary was practically that of the normal, except for a slight opacity. Microscopically the centrally located ovarian tissue showed beginning degeneration. There was coagulation of the cytoplasm and loss of definition of cell outlines. The nuclei stained very darkly, were structureless and irregular in shape. Most follicles and ova were in a fairly advanced stage of degeneration. The tunica albuginea and surface epithelium were degenerated, but not to as great an extent as the rest of the ovarian tissue. Here too the follicular degeneration was more advanced in the theca than in the granulosa.

Ovary no. 50—Vessels ligated for two days. The gross examination of this ovary showed it to be very swollen and dark red in

color. Microscopically its appearance was the same as that of ovary no. 49, except that the degeneration was further advanced; the follicles were degenerating from without inward.

Ovary no. 42—Vessels ligated for six days. Grossly this ovary was swollen and dark red in color. The surface epithelium, tunica albuginea and the tissue directly beneath them were in a state of fair preservation, but showed some disintegrative changes. The region just within this marginal band was in a state of very advanced lysis, nearly all structure being gone. The central portion of the ovary was very decidedly degenerate, but not as much so as the adjoining region. The best-preserved ova and follicles were those in the earlier stages of development, but few, if any, were well preserved. These degenerate follicles showed a very striking picture. In every case the degeneration was from without in. The thecal layers might be completely gone, even if the granulosa was only partially degenerated, the best-preserved granulosa cells being those most centrally located within the follicle. This is in direct contrast with the picture usual to follicular atresia, in which degeneration is always from within out; the theca often being perfectly preserved when the granulosa is practically gone. In a few ova a definite bright yellow pigment was seen, apparently in globules, and also found in the stroma and granulosa. A few structures which apparently were degenerated ova were also observed. A typical one was a light blue, fairly homogeneously staining sphere, with a barely recognizable nucleus. If this was an ovum it must have undergone a marked change to differ so decidedly in staining properties from that normally taken by healthy and degenerate ova in follicular atresia. A large number of blood vessels in various stages of obliteration were present.

The evidence presented by three of these cases is complicated by the presence of a hyperaemia, either acute or passive, or a venous stasis, as well as an anemia. However, it seems justifiable to conclude that when a vesicular follicle degenerates through lack of nutrition the thecal layers are the first to go, and the granulosa is the last to go. This order is the reverse of that occurring in the process of ordinary follicular atresia, in which

the first changes always occur in the granulosa, the thecal layers being the last to be affected. The coagulation necrosis was not distinctive, being, as far as was observed, similar to that occurring in any organ or part deprived of its blood supply.

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PLATE 1

EXPLANATION OF FIGURES

- 1 Degenerating ovum in a follicle with cuboidal epithelium-like granulosa. $\times 700$.
- 2 Immature ova in the germinal epithelium. $\times 500$.
- 3 Two cytoplasmic inclusions in a follicle with the granulosa entirely, and the theca largely gone. $\times 750$.
- 4 Degenerating granulosa. $\times 500$.

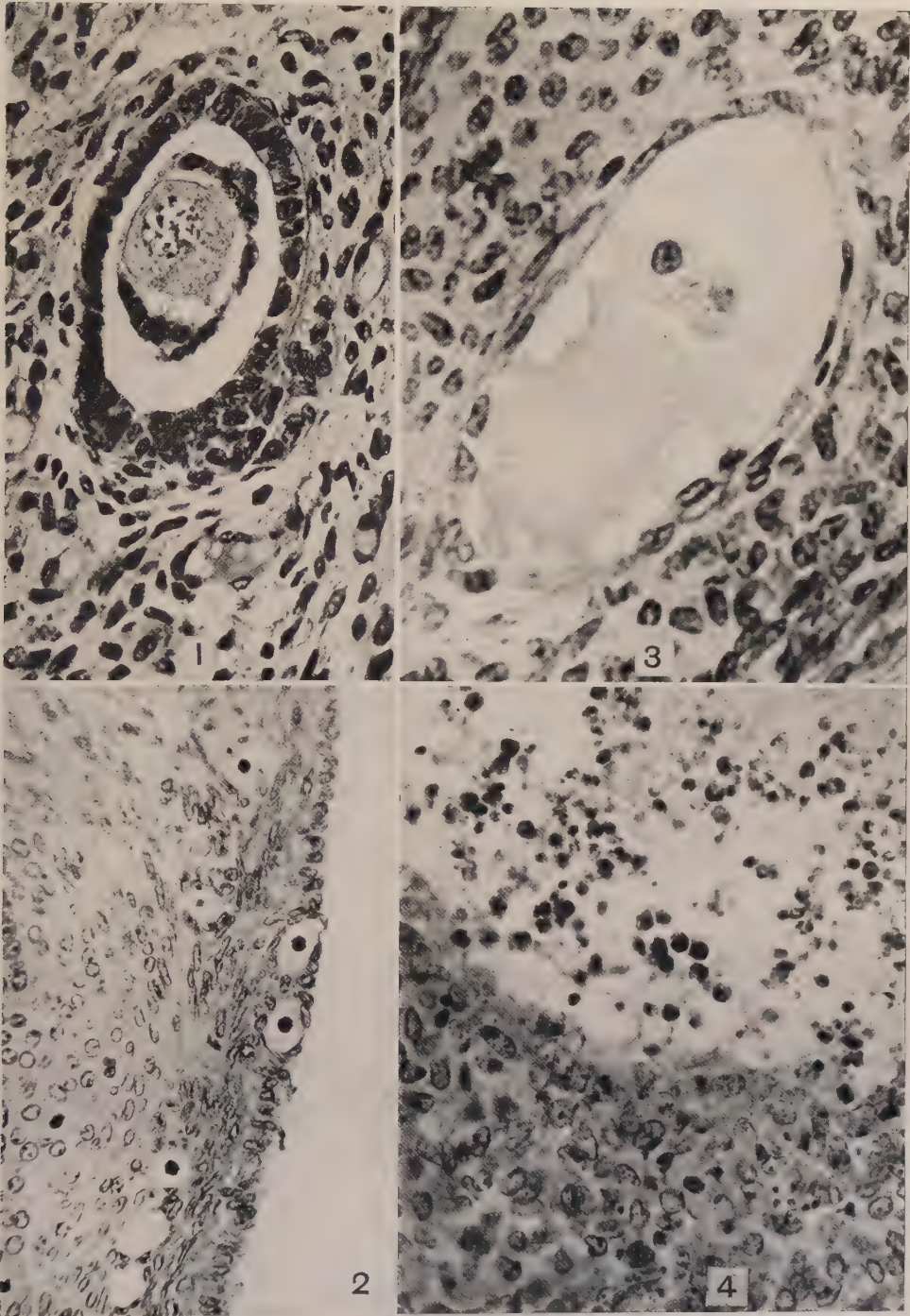


PLATE 2

EXPLANATION OF FIGURES

- 5 Spindle in a degenerating ovum. $\times 700$.
6 Degenerating follicle containing ovum of fig. 5. $\times 100$.

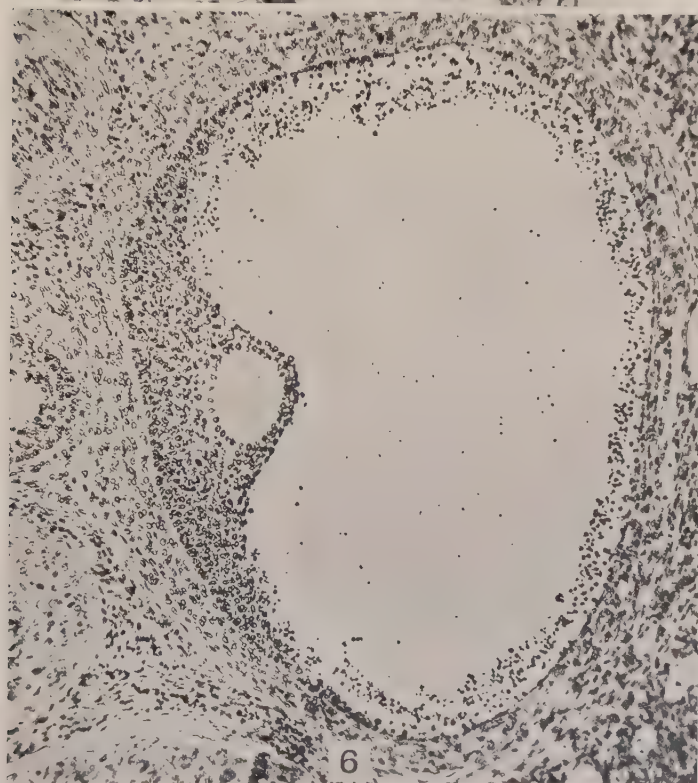
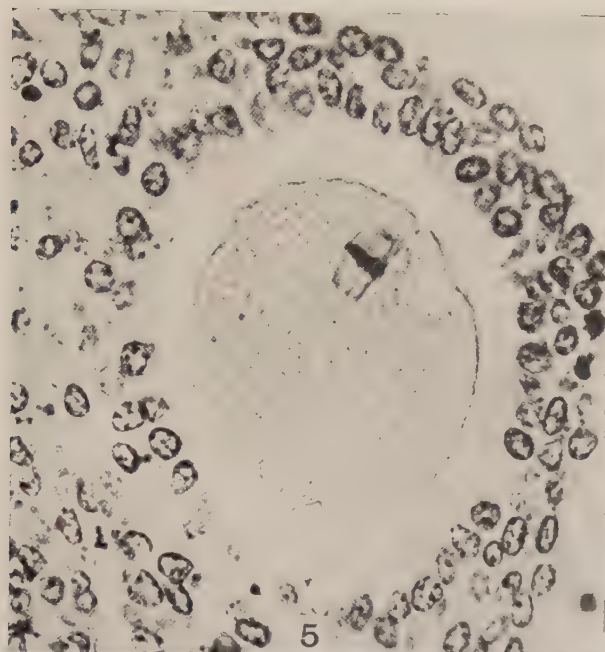
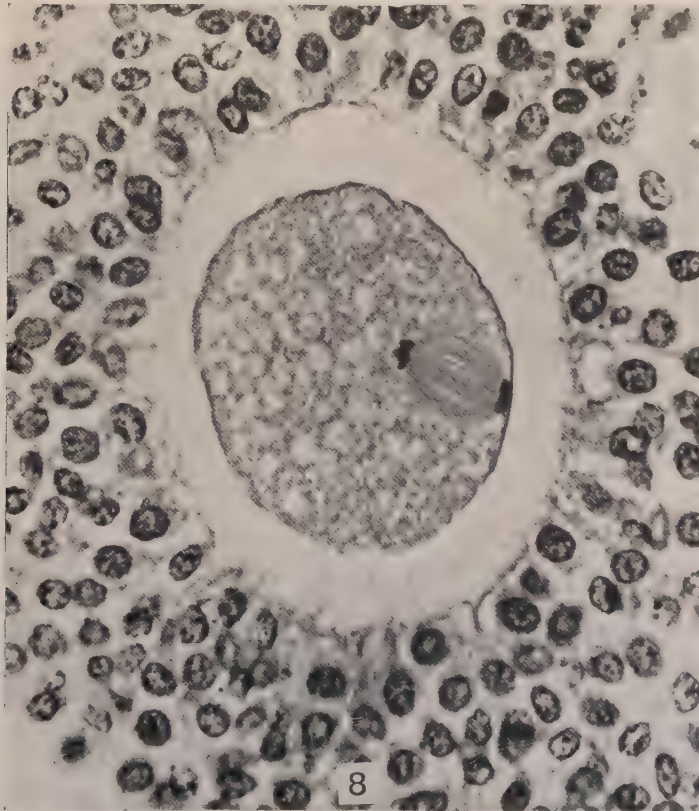
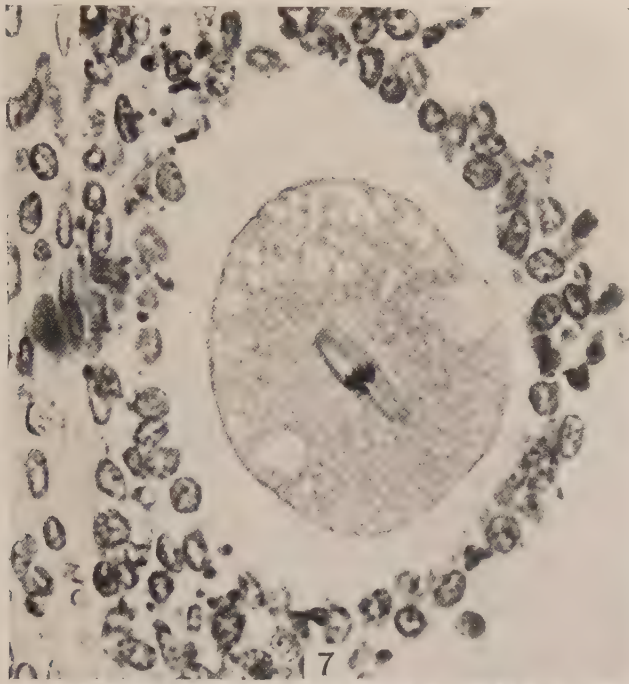


PLATE 3

EXPLANATION OF FIGURES

- 7 Spindle in a degenerating ovum. $\times$ 730.
8 Spindle in a degenerating ovum. $\times$ 730.



ON THE MORPHOLOGICAL SIGNIFICANCE OF CERTAIN CRANIO-VERTEBRAL VARIATIONS

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FOURTEEN FIGURES

Among the anatomical problems having received additional elucidation of late must be noted that of cranio-vertebral variation. The road pursued in connection with it is fairly similar for all such phenomena: it led over topographic description and speculative conjecturing to the study of ontogenetic conditions and biological argumentation. Considerable literature has been accumulated on the above subject, while only a limited number of contributions have approached its causal nature from the comparative and ontogenetic points of view. A brief historical review pointing out the principal stations may precede a few personal observations which in the light of recent investigation have acquired more decided significance.

PARTS INVOLVED

Variations in the cranio-vertebral section involve two regional factors, the occipital bone around the foramen magnum and the proximal cervical portion of the vertebral column. It is the former which, on account of its complex character, appears to be preeminently the carrier of those variations. With the rise of comparative anatomy not much more than a century ago, together with the then already established interest in the human cranium (Blumenbach, Sömmering, Camper), the scrutinizing mind of the scientist became also interested in the genetic problem of the skull. An obvious analogy between the plans of construction of vertebrae and certain groups of cranial bones

seemed to present itself. It gave rise to a theory according to which the cranium should consist of a number of vertebrae which, in the course of evolution and to meet the organic exigencies, had been transformed into the definitely shaped bones of the human cranium.

THE 'VERTEBRAL THEORY' OF THE SKULL

Proposed by Goethe and Oken, this 'vertebral theory of the cranium' aroused a great deal of controversy and for a long time remained a moot question. The recognition of three cranial vertebrae seemed to be indicated which in postero-anterior order consisted, 1) of the os occipitale; 2) the basi-sphenoid with the alae magnae and the ossa parietalia, and, 3) of the presphenoid with the alae parvae and the ossa frontalia. A fourth cranial vertebra, a nasal one, was assumed by Aeby, the constituent parts of which he supposed to be the ethmoid, nasal bones, and vomer. The cranial vertebrae, counted forward and in expression of their situation and component parts, were named vertebrae occipitalis, temporalis, frontalis, and nasalis.¹ But it was no less a scientist than Thomas Huxley who brought ponderous argument against the vertebral theory, showing by the results of wide comparative study, that the cranial bones originate and differentiate in an unsegmented skull. An homology between the cranial parts and spinal vertebrae, therefore, appeared to be improbable.²

¹ The following analytical table after Aeby ('71, p. 185) may recapitulate those conditions. It lists the constituent parts of the four assumed cranial vertebrae:

Vertebra occipitalis		Vertebra temporalis		Vertebra frontalis		Vertebra nasalis	
Corpus:	occipitale basilare	pars basilaris	Sphenoidus posterioris	pars basilaris	Sphenoidus anterioris	Vomer	
Arcus inferior:	occipitale laterale	ala major sphen.		ala minor sphen.			
	Occipitale	aquas temporalis (petrosus tympanicus)	Sphenoidus			lamina perpendicularis (labyrinthus nasalis)	Ethmoidus
Arcus superior:		occipitale superius	Parietale	Frontale	Nasale		

² In his meritorious work on the primordial cranium of the cat, Terry ('17, p. 349), reflecting on the 'vertebral theory,' says: "But the vertebral theory, notwithstanding the blows dealt it from the time of Huxley's attack to the present, has shown

METAMERY OF CRANIAL AXIS

Huxley's argumentation in opposition to the vertebral theory appears to have been accepted by the majority of scientists. Further comparative study, particularly under Gegenbaur's lead, revived the vertebral problem for the cartilaginous part of the skull. His classical study of the cartilaginous skull or chondrocranium of the selachians ('72) brought forth a new theory. While agreeing with Huxley on the autonomous anlage of the bones of the brain case and the unsegmented state of the ethmoid and partly the orbital—Gegenbaur's 'evertebral,' Kölliker's 'pre-chordal'—region, he conceived the greater posterior or 'vertebral'—Kölliker's 'chordal,' Froriep's 'pseudo-vertebral' ('82, p. 300)—portion of the skull base derived from the coalescence of vertebrae or vertebralanlagen. This theory of hypothetical cranial vertebrae appeared to be justified on the basis and in view of the previously existing visceral arches and the cerebral nerves pertaining to them, as well as of the extension of the chorda dorsalis into the cartilages later constituting the cranial axis. Gegenbaur, as Froriep ('82, p. 297) points out, thus arrived through comparative anatomical recognition at the postulate of a phylogenetic primordial segmentation in default of an ontogenetic one.

ONTOGENETIC FINDINGS

The problem of the metamery of the cranial axis received still further ventilation through ontogenetic studies, particularly of the neural conditions in embryos of ungulates, ruminants, and carnivores. It could be shown that in early ontogenetic stages

itself tenacious of life, and the thought uttered by Oken more than one hundred years ago demands deference of the worker of to-day." This statement is in just historical evaluation of Oken's morphological sagaciousness. The theory as such, although scientifically no more tenable, has shared the fate of similar elegantly shaped and easily appealing theorems, like the popularly worded "descent of man from apes," i.e., they are tenacious of life, such as the first forming an unalienable part of the scientist's cargo, the latter a stubbornly retained miscomprehension on the side of the layman. However, as regards the priority of the vertebral theory, it was Goethe who, examining a skull of an animal which he happened to pick up on the Lido of Venice in 1791, first conceived the idea of cranial vertebration.

the anlage of three or four occipital vertebrae was evident (Froriep's 'spinaler Schädelabschnitt'; '82, p. 300). Their development, however, remained rudimentary with the exception of the most caudal one, which attained more advanced stages before its definite merging with the other rudiments into the cranial axis. It received the designation by Froriep ('86, p. 133) of the 'Occipitalwirbel,' not 'an' occipital vertebra as found at times with reference to Froriep's term. The study of the primordial skull has brought forth a number of valuable contributions on that problem (Bardeen, '08; Fischer, E., '02, '03; Fuchs, '09; Gaupp, '98, '00, '05, '08; Gregory, '04, '13).

Closely interrelated with the problem of occipital metamery is that of neural metamery in the cranio-vertebral border region. However, the discussion of the different theories advanced does not lie in the scope of this article. Suffice it to mention that particularly the n. hypoglossus and its affinity to the n. vagus group on the one hand and the first cervical nerves on the other, together with the assumed successive assimilation of cervical vertebrae, gave rise to Stöhr's ('80, '81) theory of "caudal progression of the cranium." According to this theory, the homologa of the nn. hypoglossus and accessorius Willisii are not to be seen in the cerebral nerves of lower vertebrates, but in their first spinal nerves. Involved in this supposition, as has been said, is the gradual assimilation of vertebral elements into the skull. The fundamental facts from the ontogenetic point of view, however, are the absence of the n. hypoglossus in the Ichthyopsidae and its existence in the Amniota. Stöhr's theory has met with strong opposition.

Entering now upon the discussion of variations in the cranio-vertebral region, there is a group of them directly related to the genetic conditions there. It is in connection with Froriep's 'occipital vertebra' that Kollmann ('05, p. 235) speaks on its manifestation.

'MANIFESTATION OF THE OCCIPITAL VERTEBRA'

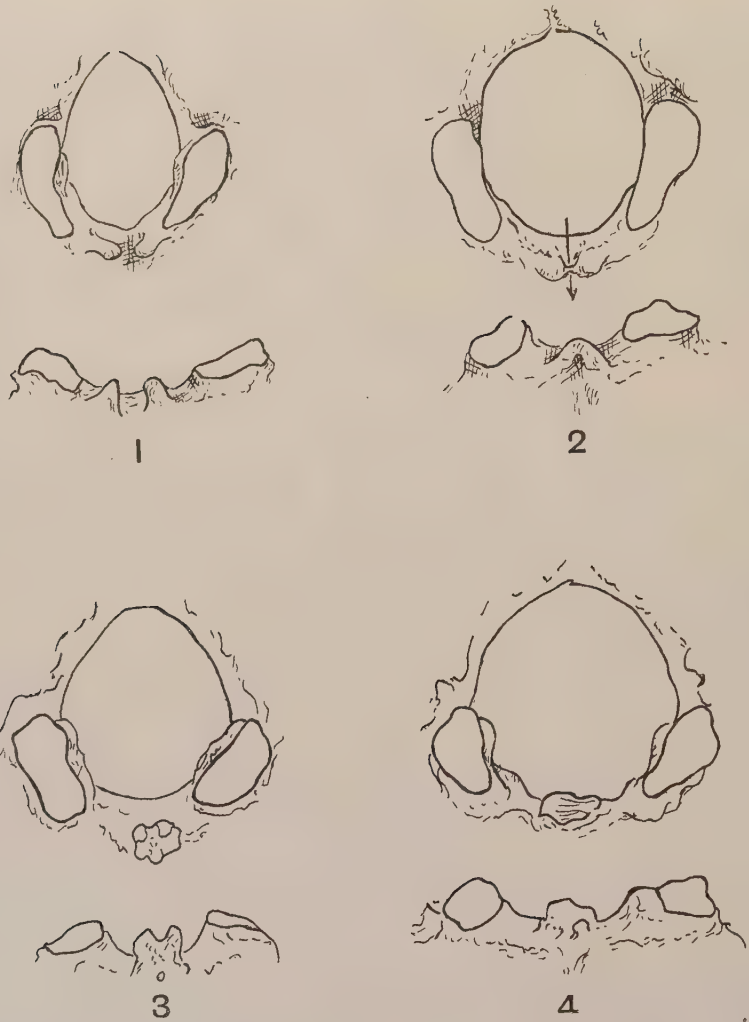
Among its indications Kollmann enumerates: *condylus tertius*; *labia foraminis magni*; *canalis intrabasilaris* (Kollmann, '05, p. 233; '17, p. 551)³ *s. intraoccipitalis* (Swjetschnikow, '06, p. 181), *incisura marginalis posterior*; enlarged *massae laterales*; *processus paracondyloideus*; divided *canalis hypoglossi*. Some of these indications have recently received veering explanation, which holds particularly true for the *labia foraminis magni anteriora* and the formation known as *condylus tertius*. Both may receive a more detailed discussion.

LABIA FORAMINIS MAGNI ANTERIORA

Of this anomaly a good instance may be seen in figure 12, illustrating that of a male Haida (no. 1606, American Museum of Natural History).⁴ It shows precondylar tubercles, also called *tubercula basilaria* which, according to Kollmann, represent the rudiments of the anterior arch of the occipital vertebra. Ending club-like with swollen ends, they do not quite unite in the median line and rise about 6 mm. above their basis, running free of the latter. The occipital condyles are elongated forward and mesially and show in their main masses medially sloping non-articular surfaces in addition to the articular ones proper. *Labia posteriora*, if any, are evident by slightly roughened edges, not joining posteriorly, but otherwise well merged with the bone. Following Kollmann's and other authors' interpretations of this anomaly, the two tubercles would be identical with the unassimilated and at the same time abnormally developed extremities of the anterior arch of the occipital vertebra. Its incompleteness in the residual state is explained as being due to the wanting corpus, of which a cartilaginous anlage occurs ontogenetically, and which,

³ As a character in the manifestation of the occipital vertebra this *canalis* is described as a homologon of the second author's *spatium atlanto-occipitale anterius* between the anterior border of the foramen magnum and the anterior arch of the atlas which, in the recent state, is filled in by the *membrana atlanto-occipitalis*.

⁴ Figures 12, 13 and 14 are, by permission of the editor, taken from the author's report not yet published, on the craniological material collected by the Jesup Expedition.



Figs. 1 to 4 Successive stages of irregular differentiation of the occipital condyles in connection with their migration lateralward, in basilar and frontal aspects. Figure 1, persistence of disposed of articular space in form of basilar or precondylar tubercles; figures 2 and 3, advanced stages of figure 1, showing mesially uniting tubercles; figure 4, concrescence of precondylar tubercles resulting in condylus tertius (after Bolk).

at an early stage, is merged with what later on forms the pars basilaris of the occipital bone.

Another explanation of the feature just described was offered of late by Bolk ('21). Instead of linking the discussed anomaly with ontogenetic conditions, Bolk sees its causation in an irregular process of differentiation. Stating the strongly ventral position of the occipital condyles at their first appearance, he asserts that during the ontogenetic development of the foramen magnum region they migrate lateralward. This would involve the disposing of articular condylar space anteriorly and compensatory apposition posteriorly. Bolk thus speaks of primary and secondary occipital condyles, stressing the point that the definite topographic conditions are not the result of the broadening of the intercondylar space, but of the actual wandering of the condyles. At times, however, this process has not yet fully come to an end in the infant stages, then signified by the persistence of the disposed of condylar parts in the form of more or less distinct non-articular ridges in mesial elongation of the occipital condyles. The medial ends of these ridges appear to be possessed of a 'positive Entwicklungspotenz' which causes them to enlarge and acquire shapes characteristic of the tubercula basilaria. If this view should prove correct, a simple and plausible explanation would have been found and the theory of ontogenetic residua of the occipital vertebra, at least in a number of cases, frustrated.

CONDYLUS TERTIUS

But Bolk, following up the thriving tendency of mesially progressing tubercula, lays also to it the formation of condyli tertii. The successive stages of advance toward, and merging in the median line are illustrated in text figures 1 to 4, after Bolk ('21). Figure 1 represents a stage corresponding to our own figure 12, which latter is a particularly fine case. Figures 2 and 3 still show in the accomplished coalescence traces of the original state in the medial perforation, an arrow marking that state in figure 2. In addition to the perforation the two tubercles are still evident in figure 3. A true condylus tertius is to be seen in figure 4, still showing, at its anterior side, the vestiges of its derivation.

It arises at the anterior margin of the foramen magnum and has an articular surface for the articulation with the odontoid process of the epistropheus. Figures 1 to 4 illustrate the described conditions in basilar and frontal aspects. Characteristic of the condition of figure 4 is our own case of a true condylus tertius, as illustrated in figure 13. It is that of a male Yakima (no. 4334, American Museum of Natural History). The transverse and longitudinal diameters of the accessory condyle amount to 14 mm. and 12 mm. It rises about 4 to 6 mm. above its basis and is divided by narrow channels from the true condyles. The extracranial basilar as well as the intracranial clivus surfaces are continuous with the anterior and posterior walls of the third condyle, and it is only the first one which is slightly set off against the pars basilaris. The articular surface is somewhat concave and has a roughened aspect, is almost horizontally directed, and only mildly rising from below and before to above and behind. Other indications of the occipital vertebra are only indistinctly to be perceived. The occipital condyles, as shown by the illustration, are transversally flexed in a distinct ridge. The two facets form angles between themselves of about 115° on the left, and 128° on the right side. There are no processus paracondyloidei nor a bipartition of the canalis hypoglossi. The labia foraminis magni posteriora are slightly developed without joining posteriorly where they stay short of the median line for about 1 cm. on either side.

Bolk, however, does not deny manifestations of the occipital vertebra, as shown in another of his papers of recent date ('22). According to him, unassimilated parts of the anterior arch of the occipital vertebra do not appear as labia or tubercula, but as ventrally and mesially directed osseous thorns or laminae. They are continuous with the anterior ends of the occipital condyles, following the curve of the anterior border of the foramen magnum, but remain free of it. Figures 5 and 6 illustrate these conditions. Bolk infers that these rudiments are not derivations of the corpus of the occipital vertebra, but rather its haemapophysis. But he calls attention to the fact well known to embryologists that in ontogenetic stages the chorda dorsalis, after leaving the odontoid

process forms a swelling. The skeletogenic sheath of the chorda surrounding this swelling does, not infrequently, show distinct indications of calcareous deposit, and if the swelling is preserved at times, it crowns the odontoid process as a tiny bud. Under further histological differentiation it may completely ossify and may then be found joined to the anterior border of the foramen magnum in the shape of an osseous point or tubercle. It would not be entirely out of the question that the chorda-swelling represents the body of the occipital vertebra—a suggestion also made by Bolk himself (p. 158). It would imply analogous processes of differentiation for both the occipital and the first cervical vertebra (atlas), i.e., an isolation of their corpora and the formation of hypochordal arches which in the atlas is identical with the arcus anterior. His findings are thus in accord with the previously established ones by Lachi ('85), Chiarugi ('95), and Weiss ('01).

OSSIFICATION OF LIGAMENTS

Following up these deductions would lead to the assumption of a genetic derivation likewise for certain peg or thorn-like forms of condylus tertius, described generally as being due to the ossification of ligaments. That variations occur in the latter, too, is shown by Bolk ('21, p. 342), who exemplifies three different conditions as represented in figures 7 to 9. The first one amounts to just a tubercular protuberance, which, in figure 8, is of enlarged size besides being distinctly budded. An interesting but rare case is that pictured in figure 9, whose articular surface is cup-like, broadened and depressed. It very nearly resembles what might be called a true third condyle, in the sense of figure 4 and of figure 13, if it were not for the comparatively slender stem upon which the articular surface sits and which in the other case is absent or rather replaced by a broad basis. Figure 14, shows the first-named anomaly in a Salish skull (no. 1762 ♂, American Museum of Natural History), due apparently to the ossification of the ligamentum apicis dentis epistrophei, in connection with that of the ligamentum cruciatum atlantis or, better, its crus superius. The osseous eminence is situated medi-

ally and projects about 3 mm. into the lumen of the foramen magnum. It is fairly cylindrical and about 2.5 mm. thick. There is a variability of size and form to be marked in this simplest form of ossification. But what excessive sizes there might be attained is shown in a case described by Schlaginhaufen ('07), which is illustrative of the budded form of figure 8. Schlaginhaufen's case may be seen in redrawn outlines of his illustrations in figures 10 and 11, which represent the external and intracranial aspects. The anomaly was found in the skull of a Pak-pak-Battak. The length amounts to 10 to 13 mm., the latter figure applying to the intracranially situated part of the basis of the budded eminence. Width and thickness are approximately 6.2 mm. to 4.6 mm. near the basis; 3.7 mm. to 2.4 mm. in the constricted middle, and 4.3 mm. to 1.7 mm. at the end. The author mentions also an articular surface at the thinning underside of the clubbed end. The width measurements on the other hand are expressive of the constriction producing that end.

DISCUSSION

Ossifications like these would come under the caption of non-genetic causation, i.e., not as ontogenetic residua, but as characters acquired *intra vitam*. A decision, then, in individual cases as to their nature must needs be exceedingly difficult and uncertain. Similarly uncertain is the term itself, since any anomalous growth from an articular impression to a well-developed condyle is called *condylus tertius*. The views have not changed very much since the paper by R. Havelock Charles ('93, p. 15), in which he says: "The 3rd condyle . . . may either be an articular depression, a single and medium tuberosity with an articular facet, a bilateral facettted tuberosity or, lastly, an unilateral or bilateral non-articular tubercle. It may articulate with either the anterior arch of the atlas, medially or laterally, or with the odontoid process. It may be developed in the suspensory ligament, in the median occipito-atloid ligament, or in the anterior lateral occipito-atloid ligament." Thus the term of *condylus tertius* would include genetic and non-genetic articular formations, among the latter the ligamentous ossifications. A



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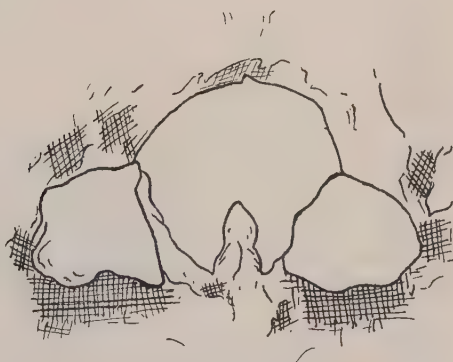
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Figs. 5 and 6 Spicula and lamina formations as residual manifestations of the hypochochordal arch of the occipital vertebra (after Bolk).

Figs. 7 to 9 Three different forms of ossification of ligaments at the anterior border of the foramen magnum: tubercular, clubbed, cup-like (after Bolk).

Figs. 10 and 11 Ossification of ligamentum apicis dentis epistrophei of extraordinary size in basilar and intracranial aspects (after Schlaginhaufen).

distinction again based on articulation or non-articulation alone—condylus really standing for joint—would be of no avail, since articulations are likewise met with in cases due to ossification. They also occur as ‘articular depressions,’ as E. Havelock Charles correctly remarks in his enumeration of condylar possibilities. One of our own cases (fig. 14), not hitherto referred to in this connection, confirms this statement. The illustration shows an articular area around the anterior margin of the foramen magnum anteriorly bordered by a continuous ridge connecting the ventral ends of the occipital condyles. This may be in further illustration of Bolk’s theory of a thriving tendency (‘positive *Entwicklungspotenz*,’ p. 17) manifested by those ventral ends under special conditions to be remarked on presently. There is first, however, another factor to be mentioned, namely, the articulation of the anterior atlantal arch with the basilar area around the foramen magnum. Although to be considered an anomalous condition, it is nevertheless in reminiscence of ontogenetic conditions when in the first stages of differentiation the anterior border of the foramen magnum is still squeezed in between the anterior arch of the atlas and the odontoid process of the second cervical vertebra. Its extrication thence takes place with the development of the condyles, their migration and rising, in brief, their springing into function. Any irregularity in this process will naturally involve other parts. Affording special conditions in the above sense, these could thus be imagined to work as stimuli upon the thriving tendency referred to repeatedly and which Bolk apparently assumes as existing *per se*. The center of growth is here of course the synchondrosis intra-occipitalis anterior whose definite ossification may likewise be

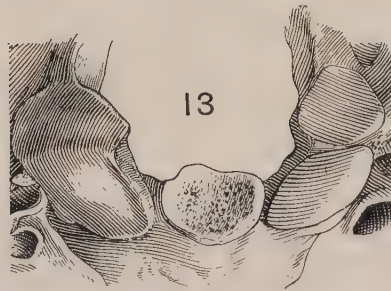
Fig. 12 Basilar or precondylar tubercles in a male Haida (no. 1606, American Museum of Natural History). Compare figure 1, page 6. Natural size.

Fig. 13 Condylus tertius in a Yakima (no. 4334, American Museum of Natural History). Compare figure 4, page 6. Natural size.

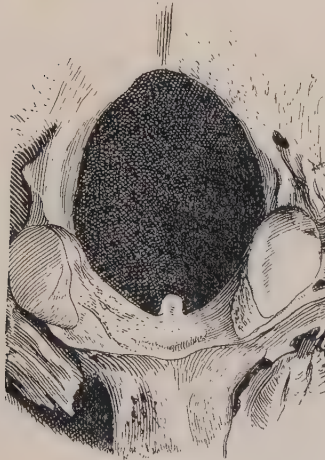
Fig. 14. Ossification of ligamentum apicis dentis epistrophei in a skull from Vancouver, B. C. (no. 1762, American Museum of Natural History). Compare figure 7, page 11. Natural size.



12



13



14

influenced by the same stimuli. It could furthermore be imagined that another group of stimuli resulting from the changes of posture and gait would, in the course of phylogenetic evolution, have a bearing on the problems of monocondyly and dicondyly. But their discussion is beyond the scope of this article.

Summing up, it appears evident that the causation and derivation of certain variations in the cranio-vertebral region is not yet fully recognized. Causation and derivation may be genetic or non-genetic, variations reversive or newly acquired. Investigation has logically arrived at a station where broad comparative and ontogenetic methods are preparing the field from which definite recognition may be reaped.

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PLANTAR EPIDERMAL CONFIGURATIONS IN LOW- GRADE SYNDACTYLISM (ZYGODACTYLY) OF THE SECOND AND THIRD TOES

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THIRTY-FIVE FIGURES

INTRODUCTION

There are certain fundamental problems within the field of epidermal ridge configuration which cannot be solved at present through phylogenetic studies, and it seems improbable that the nature of the material will permit experimentation. However, a fruitful source of data is provided by the various developmental digital defects, notably hyperdactylism, syndactylism, and ectrodactylism. It is believed that data obtained from these naturally induced alterations of structure bear upon such important issues as the mutability of skin patterns, the sources of their variation, and even the principles underlying the production of pattern forms.

Epidermal patterns have been ignored almost without exception in the recorded descriptions of congenital defects of the digits. Only a few cases of hyperdactylism have been studied with reference to ridge patterns (Wilder, '04a; Danforth, '19; Cummins and Sicomo, '22). The number is too limited to indicate definitely the bearing of the findings, which, nevertheless, are so suggestive as to emphasize strongly the desirability of further studies upon this and other defects. No observations of the patterns in syndactylism have been noted by the writers, with the exception of a casual reference by Danforth ('21) who states, "In some cases at least the friction ridges of the syndactyl fingers are continuous."

Low-grade cases have been selected as an introduction to the study of patterns in syndactylism, since they present a condition in which the individual identities of the fused digits are maintained, despite their aberrant skin relations. The present paper is confined to this type as it occurs in the foot, and particularly the cutaneous form. Materials illustrating the remaining types of syndactylism, as well as other defects, are being collected and studied for future report.

The current nomenclature of the types of congenital syndactylism is not uniform. Therefore, it is necessary to present the following brief classification, which is in part original, but largely a composite based upon several writers (Annadale, '66; Beno, '86; Kümmel, '95; Herran, '98; Weidenreich, '21-'22).

A. *Low-grade* (the zygodactyly of Weidenreich): Digits united only by skin; their general identities not obscured; heightened degree manifested in increased distal extent and thickness of skin union, closer approximation of the affected digits, and in the involvement of digits other than those primarily affected (second and third of the foot, third and fourth of the hand).

1. *Membranous*: Union merely a notable extension of the usual interdigital membrane; involved digits not closely approximated by the union; varies individually not only in distal extent, but also in breadth, thickness, and rigidity; properly, only examples of this class may be called 'webs'; illustrated by Weidenreich, figure 44.

2. *Cutaneous*: Union effected by direct continuity of both dorsal and plantar (or palmar) skin of the digits; involved digits closely and inseparably approximated throughout the extent of union; varies individually in distal extent only; unions appropriately called 'skin bands'; an advanced degree of this type illustrated in figure 3.

B. *High-grade*: A more heterogeneous group than A, comprising all stages of digital amalgamation more advanced than A, with progressive merging of their identities; fusion commonly more advanced distally than proximally; digital localization as in A.

1. *Fibrous*: Advanced union with digits more closely merged than in cutaneous syndactylism, but excluding those cases which fall under the next head.

2. *Osseous*: Digital bones fused in lesser or greater degree. (Here would be placed by some authors cases exhibiting total absence of one or more digits, but such cases more appropriately are designated ectrodactylism.)

Digital fusions usually are exhibited bilaterally. The numerical occurrence of low-grade syndactylism is a matter of interest. In most individuals the membrane between the second and third

toes is longer than the others (Kümmel, '95; Weidenreich). When this membrane is exceptionally prolonged (as in our case 246) it is, ordinarily, thin and lax as in the normal, but in three of our subjects (13, 17, and 37) it is thick and rigid, binding the toes rather firmly against the distal border of the sole. Schurmeier ('22) gives exact data on the incidence of cutaneous syndactylism. In the examination of 20,000 draftees he finds eight cases, invariably involving the second and third toes. Since the number of individuals questioned or examined in the writers' search for cases of digital defects was not recorded, we have no basis for comparison with Schurmeier's ratio.

No developmental implication is to be attached to such terms as fusion and union. They bear significance only with regard to structural relations, for, as both Danforth ('21) and Weidenreich point out, low-grade syndactylism results from the failure of embryonic digits to develop separately rather than from a secondary coalescence. Weidenreich contends that low-grade syndactylism is not pathological, but is an atavistic expression of the primitive linkage or 'zygodactyly' of the digits most commonly involved. While admitting a probable ancestral syndactylism, the explanation of low-grade syndactylism as a simple atavism may not be a sound one. Evidence which suggests that cutaneous syndactylism is pathological is the fact that it forms the first step of a graded series of digital fusions, a series which is completed by the undeniably pathological high-grade forms. Even some examples of the membranous type may be added to the lower range of the series if the exceptionally long interdigital membranes and the thick rigid ones represent intergrades between the normal digital union and cutaneous syndactylism. Suggestive comparisons may be drawn between syndactylism and hyperdactylism, indicating that closely allied factors, operating in digital zones which are developmentally plastic owing to their phylogenetic history, may be responsible for the diverse defects. While in low-grade syndactylism there is a simulation of an ancestral type and no such predecessor can be postulated for hyperdactylism, and in spite of the antithesis of their structural expressions, the two defects possess certain

characteristics in common. Both occur occasionally in monsters which owe their genesis to such a factor as retardation of developmental rate in an early embryonic period. Both are rather consistently, but not infallibly, selective as to the digits affected. Both are heritable, but expressed with occasional variations in localization and degree. Thus, as in a family described by Newsholme ('10), only the right hand of one individual is affected, although the other syndactyls have fusions joining the second and third toes. The fraternal syndactylism of 26 and 27 and of 31 and 34, reported in this paper, illustrates a different sort of variation in localization. Both 26 and 31, from different families, show the condition bilaterally while the sister of each exhibits it on one foot only.

MATERIAL

Twenty syndactyls have been studied, sixteen of them having the cutaneous form and four the membranous form of union. Although they are syndactyls by definition, individuals with simply longer interdigital membranes have not been included, except where the union presents some feature of particular note. Thus, 13, 17, and 37 have received attention because of the thickness and digital relations of their membranes, and 246 has been included because of a noteworthy prolongation of the union. In table 1 the cases are listed and briefly characterized, each being designated by a number which refers to the sole prints in our collection. The value of several of the cases is enhanced by the possession of sole prints of other members of their families. Prints of over 250 individuals rated as non-syndactyls or possessing only slightly attenuated and thin interdigital membranes have been utilized for comparison with the syndactyl material.

The writers are indebted to Prof. H. H. Wilder for the use of a number of sole prints from his collection and for information concerning the families represented. Each of these cases is distinguished in table 1 by the presence of a second number, his accession number, followed by 'W,' our number then referring to our tracings from the same prints.

METHODS

Formulation is an adjunct to any descriptive account of the patterns existing upon the palmar and plantar skin, inasmuch as their wide range of variation would render the otherwise necessary verbal description voluminous and intricate or require illustrations too numerous to be practicable. In formulae the most complex configurations may be presented concisely, and so accurately that reconstructions based upon them approximate the original patterns in all their larger and fundamental features.

Owing to their general use as a means of personal identification, the apical patterns of the fingers have received most attention. Consequently their formulation now is in a more nearly perfected state. Here the situation is relatively simple in that only one pattern occurs upon each finger, while for the palm and sole the formula must embrace the interrelationships of the several patterns as well as the type of each. Wilder has elaborated a method of formulating palmar patterns which is comprehensive enough to permit their reconstruction, although the formula in its simplest form is expressed in only four symbols. The greater complexity and individual variation of the sole patterns render their formulation a more difficult matter, and in addition to these natural obstacles there is the further disadvantage that critical areas at the distal edge of the sole often cannot be made to appear in the print.

For descriptive purposes, and for subsequent formulation, the sole presents two sets of features, either of which may be emphasized. There are, first, the triradii, which vary in occurrence, position, and relations in individual soles. Schlaginhaufen ('05) devised a scheme in which each triradius is assigned an empirical number, and emphasis is accorded to them rather than to the patterns with which they are related. Wilder ('16) objects to the usage, as follows: "This method is thus a method of description rather than a method of formulation, and although in many ways convenient, it depends upon absolutely exact homologies, which, in our present state of knowledge, is not possible." The alternative feature consists of the patterns themselves. In this case the triradii and radiants extended from them

are secondary, serving only as boundaries between the primary areas. Beginning with 1902, Wilder's successive publications upon the subject show the progressive development of a method in which the patterns are emphasized in the construction of a descriptive formula.

For the purposes of the present account and of others in preparation which likewise deal with plantar patterns Wilder's formulation is insufficient. In two ways it fails to meet the need of a study devoted to the region at the distal border of the sole. The first of these, the omission of the relations of patterns to the interdigital spaces and to each other, is an intentional adaptation to the technical difficulties which arise in printing completely the region at the bases of the toes. While its incompleteness in this respect is an advantage for the intended routine use in personal identification, the neglect of features in the region which happens not to print readily renders the formula inadequate for complete description. Although these features are dependent upon complete prints, such prints are not impossible to obtain. When the entire tread area can be impressed, as in children especially, they are brought out with ease in one print. In dealing with those individuals whose soles do not permit complete impressions the details may be printed separately. The second deficiency in Wilder's formulation is that certain areas between patterns, which are of uncertain morphological significance, are not included. It is necessary, then, to supplement Wilder's formula by the inclusion of all regions and by a definite indication of the relations of the patterns to each other and to the interdigital spaces.

The word 'formula,' as used in application to the method described below is inappropriate, in the sense of Wilder's usage of the term. However, it will be applied for the sake of brevity. The elements of the sole patterns indicated in the formula are: the hallucal pattern, the first, second, and third interdigital patterns, the triradii and radiants associated with them, and fusion of the digital areas. The hypothenar, thenar, and calcar patterns bear no relation to the present study; consequently, they have not been included in the formula. Each element of

the sole patterns is indicated in the order named above, and each detail of a given element is noted in the order of occurrence, beginning at the tibial border. The nomenclature of the patterns and other elements follows that of Wilder, and except where additions have been necessary the symbols are equivalent. In order to ensure complete description, the areas marked off by even prolonged extensions of the radiants are stressed, though often at the expense of their true morphology. Comparison of the figures and the formula of the soles which are illustrated, as listed in table 1, will render the following tabulation of symbols more intelligible.

Explanation of the symbols used in the plantar formula

I. *Primary symbol* of the hallucal pattern: O, U, or W, carrying the same significance as in the interdigital patterns, excepting that the U applies to any pattern with recurved ridges at one border, and without regard to the direction of this border, the direction of the opening being indicated by an appropriate exponent.

Ia. *Exponents* which qualify the primary symbols of the hallucal pattern: Like exponents bear the same significance as those applying to interdigital patterns. W may be qualified by special exponents, as sm, TL, according to the usage of Wilder and Wentworth ('18).

Ib. *Prefixes* which qualify the primary symbols of the hallucal pattern, enclosed in parentheses: A, B, and C, referring to the distal, tibial, and fibular triradii, respectively, of the hallucal pattern. The symbols stated in the formula are those of the triradii which are absent, following the method of Wilder. In interpretation of these triradii there may be occasional grounds for uncertainty, especially with reference to C. In the cases of A and B it is sufficient for descriptive purposes to rely upon the positions of the triradii with reference to the pattern, i.e., the pattern is proximal to A and on the fibular side of B. C is on the fibular side of the pattern, but a question of whether a triradius in this relation represents C or a proximal triradius frequently arises. To meet this difficulty the writers have assumed that to be C it must either join or lie tibial to the proximal radiant of the first digital triradius. When the first digital triradius is absent, and no fork is present in the corresponding position, which might be extended to determine a boundary, the triradius is interpreted as C, providing its distal radiant does not join the proximal radiant of the second digital triradius.

II. *The symbol X*, for an area which has not been assigned to any one of the patterns, lying between the hallucal pattern and line A, when triradius C of the hallucal pattern is present. It is always an open field, and in formulation is followed by the same exponents which apply to the open fields of interdigital areas.

III. *Primary symbols* of the interdigital patterns, indicating the configuration of their ridges; any one interdigital pattern is represented by one primary symbol only if it occupies the entire space between two neighboring triradii, or by two or even three primary symbols if radiants enter and cleave it: O, an open field, that is to say, a series of ridges coursing more or less parallel, and with no recurved ridges. U, a loop, with a closed end formed by recurving ridges, the closed end being directed proximally. Ω , a loop, with a closed end formed by recurving ridges, the closed end being directed distally. W, a whorl, a concentric series of ridges.

IIIa. *Exponents* which qualify the primary symbols of interdigital patterns, denoting the relations of these patterns to the tibial and fibular borders of the sole, to the interdigital spaces, and to each other: t and f, indicating respectively, that the proximal opening of an O, U, or separated (tangential) portion of a W is directed to the tibial or fibular border of the sole.

1, 2, 3, and 4, indicating the respective interdigital spaces, to designate the primary distal opening of an O or U. In case an O is noted as having a primary distal opening, and with no proximal opening indicated (as O 3), the area is completely occupied by the secondary openings of other patterns.

a, b, c, and d, indicating the first, second, third, and fourth interdigital spaces, respectively, to designate the secondary or distally recurved opening of an O, U, Ω , or separated (tangential) portion of a W. When preceded by the Roman numeral indicating an interdigital area, the intent is to show that the secondary opening is attained through the territory of the area indicated by the numeral.

Hal I, II, and III, indicating the hallucal and respective interdigital areas, designating that an O or U enters the area named in the exponent, appropriating it completely if no further exponent follows, or only partially if a secondary opening is stated to occur.

cl, indicating proximal closure of an O or U by the fusion of radiants from a proximal triradius (d), from the digital triradii (A, B, C, and D, respectively), or from the tibial, distal, or fibular radiants (m, n, and o, respectively) from C of the hallucal pattern.

IV. *Digital triradii* are numbered 1, 2, 3 and 4, in order beginning medially.

Absence of a triradius indicated in formula by the minus sign (—) preceding the number of the triradius.

A fork, in the position of a triradius, indicated in formula by the caret ($\wedge$), preceding the number of the triradius which it represents.

A convergence, in the position of a triradius, indicated in formula by the symbol (λ), preceding the number of the triradius which it represents.

When the formula contains no reference to digital triradii it is to be understood that the full complement is present.

V. *Proximal triradii* are indicated by d, dd, according to the number present.

May be qualified by exponents A, B, C, or D, indicating that a radiant joins the digital radiant named in the exponent.

May be qualified by exponents I, II, or III, indicating that a radiant cleaves the pattern named in the exponent.

When the formula contains no reference to proximal triradii it is to be understood that none are present. See Ib.

VI. *Fusion of digital areas* is indicated in formula by $2 + 3$, $2 + 3 + 4$, etc., the respective digital areas being indicated by number.

DESCRIPTION

Nature of the webs and skin bands

The webs and skin bands merit especial attention with regard to hair distribution and epidermal configurations. Danforth ('21) discusses the subject of hair distribution upon the normal digits. It is of interest to note that the dorsal epidermis of both webs and skin bands does not bear hair, even though hair is present at the same level upon the adjacent digits. Epidermal ridges invariably occur on the plantar surface. Upon the thin and lax interdigital membranes the course of the ridges is practically longitudinal, but upon the thicker interdigital membranes and all skin bands their course is transverse, and individual ridges can be traced uninterruptedly across the entire breadth of the union. Further details concerning the ridges in this region are noted below, in the discussion of basal transverse ridges. Whether the dorsal aspect of the skin union is holomogized with the dorsal surface of the embryonic interdigital tissue or with the sides of the digits, the absence of hair is not unexpected, since neither region carries hair-forming potencies. The occurrence of epidermal ridges is in harmony with either homology which could be proposed, comparing the plantar aspect of the skin union with either the plantar surface of the embryonic interdigital tissue or the more ventral portions of the sides of the digits.

Epidermal configurations of the digits

The configurations of the toes may be treated under three heads: 1) apical pattern, 2) basal transverse ridges, and, 3) digital area.

1. Only in one case (202, figs. 1 to 5, inclusive) is the distal extent of the skin union sufficiently great to modify the apical patterns. In this case it is obvious that each of the fused digits bears a whorl, together with the elements associated with this type of apical pattern when it exists upon a normal digit. Associated with their abnormal contiguity, there is a marked displacement of certain elements with the formation of a new one. As will be noted in the figures, aberrant positions are occupied by those portions of the patterns which would have occurred normally on the apposed surfaces of the second and third toes. By analogy of relations, but probably not following their true homologies, the shifted elements are interpreted as follows: *a*) The apposed margins of the whorls are shifted, as evidenced by the extralimital position of their opposite margins. Such patterns usually are placed symmetrically. *b*) Triradii which lie normally upon the apposed sides of the digits are found at the extreme distal end of the digital mass, together with, *c*) the related basal transverse ridges, which now form a narrow area of vertically coursing ridges between the triradii. *d*) The courses of the arciform ridges distal to the whorls are limited, with reference both to extent of individual ridges and to the area occupied by the series. *e*) The new element is a triradius lying at the point of junction of the two whorls with each other and with the basal transverse ridges of the skin band. The occurrence of the new triradius recalls the accessory degeneration triradii noted by Miss Whipple ('04) and Schlaginhaufen, in apical patterns of the toes. According to the former writer, an originally simpler pattern may, through extension into a sinuous figure or separation into isolated loops, establish accessory triradii, which are "not originally present in the typical scheme but formed incidentally in the process of degeneration of the pattern." Both the accessory degeneration triradius and the triradius of the fused digits appear in response to novel junctions of ridges, with digital fusion accountable for such junctions in the latter case.

2. The basal transverse ridges course transversely across the skin band or web (unless, as in 246, the web is thin), many of them without interruption. Naturally, the number which is

thrown into continuity is subject to variation in accordance with the distal extent of the skin union. A triradius is inconstantly present at the distal margin of the band or web, its presence and degree of definition being determined by the difference in direction of the basal transverse ridges actually upon the skin union and those upon the free portions of the toes. The occurrence of the triradius bears no relation to the extent of union. In 246, the exception noted above, the basal transverse ridges turn distally along the margins of the web, those of the two toes being separated by the intervening opening ridges of the first interdigital area and of the neighboring unfused digital areas. This represents the typical relationships of the normal, when digital areas are not fused.

3. With the exception of the same case (246), fused digital areas are associated constantly with syndactylism, as is shown in table 1. Where digital areas are fused, in all cases save one (245), the extent of fusion is equal to the extent of syndactylism; in the exception only the second and third toes are joined, while the second, third, and fourth digital areas are fused. Most closely related to the unfused condition is the situation where at least two digital areas are approximated mutually, with all their ridges directed into the related interdigital spaces. With progressive stages of true fusion the ridges which lie within the most proximal portions of the involved digital areas become continuous with each other, leaving only those in the distal portions related to the interdigital space or spaces. Coincident with fusion, there is an apparent proximal migration of the digital areas, even to the extent that the boundary between them and the neighboring interdigital patterns may be obliterated, in case the latter are intergrading types between whorls or inverted loops to open fields or loops. The merging is marked especially in those cases which show extreme deviations of the first and second interdigital patterns with their openings in distant interdigital spaces. Distally, there may or may not be a triradius marking the point where the strictly transverse ridges of the completely fused portions come in contact with the distal ones which extend to the interdigital space or spaces.

TABLE 1

The plantar configurations (excluding hypothenar, thenar, and calcare patterns) of twenty syndactyls and members of their families; also showing the configurations of several members of a family in which there is a strong inheritance of fused digital areas, with occasional traces, but no marked cases of syndactylism (210 to 214, incl.). E, right; L, left. Underlining of R and L indicates syndactyl feet.

No. and sex	Character of digital union	Relation-ship	Foot	Hallucal area	X	First interdigital area	Second interdigital area	Third interdigital area	Digital areas fused	Digital triradii absent	Proximal triradii
13 M	thick webs between toes 2 and 3		<u>R</u> <u>L</u>	(C)W ⁰ O ¹ t (C)W ^a O ¹ t		O ¹ t O ¹ t	O ^{3t} U ¹ O ^{3t} U ¹	O ⁴ t O ⁴ t	2+3+4 2+3+4	-1 -1	
17 M	thick webs between toes 2, 3, and 4		<u>R</u> <u>L</u>	(A)U ¹ O ¹ t (C)W ^{smat} O ¹ t	I ^{II} c	O ¹ III d U ¹ (U ¹ c)(III d)	U ¹ U ¹	O ⁴ t O ⁴	2+3+4 2+3+4	-1, -2, -3 -2	d ^I
20 M	skin bands between toes 2 and 3; web between 3 and 4		<u>R</u> <u>L</u>	(B)U ¹ O ¹ t (BQ)U ¹ O ¹ t		O ³ II O ^{3t}	W W ^{III} d	U ¹ O ⁴ t	2+3+4 2+3+4	-2 -2	d ^{III}
217 M		Brother of 20	R L	(BQ)U ¹ O ¹ t (C)W ¹ O ¹ t		U ¹ U ¹	U ³ O ^{3t} O ³ III d U ³	O ⁴ t O ⁴ t	2+3 2+3		d ^{BC} d ^{II} III
26 M	skin bands between toes 2, 3, and 4		<u>R</u> <u>L</u>	(A)U ¹ O ¹ t (A)U ¹ O ¹ t		O ¹ III d O ¹ f	U ¹ O ¹ III d U ¹	O ⁴ t U ⁴	2+3+4 2+3+4	-1, -2 -1, -2	d ^D III
27 F	skin bands between toes 2 and 3	Sister of 26	R L	(AC)U ¹ O ¹ t ?		O ¹ t O ¹ t	O ^{3t} O ^{3t}	O ⁴ t O ⁴ t	2+3 2+3	-1? -1?	
29 F		Mother of 26 and 27	R L	(A)U ¹ t (A)U ¹ O ¹ t	I ^{III} d	O ² II c U ^{III} d O ¹ III d	U ³ W ^{III} d	O ⁴ O ⁴			
30 M		Father of 26 and 27	R L	(A)U ¹ O ¹ t (AC)U ¹		O ² III d O ² t	U ³ O ^{III} d U ³	O ⁴ t O ⁴			d ^{BD}
28 F		Sister of 26 and 27	R L	(A)U ¹ O ¹ t (AC)U ¹ O ¹ t		O ² III d O ² t	U ³ O ³ III d U ³	O ⁴ t O ⁴			d ^{BD}

31	F	skin bands between toes 2 and 3		R	(A)U'O't		O ¹ III d	U ³	O ⁴ t	2+3	-1	
				<u>L</u>	(AC)U'		O ¹ t	O ³ III d U ³	O ⁴ t	2+3	-1	d ^B
34	F	Skin bands between toes 2 and 3	Sister R of 31	<u>R</u>	(AC)U'O't		Π^2 O ¹ t	U ³	O ⁴ t	2+3		d ^{BC}
				L	(AC)U'		O ² t	U ³	O ⁴ t			d ^{BC}
32	F		Mother of 31 and 34	R	(A)U'O't		O ² III d	U ³	O ⁴ d m			
				L	(C)W'tt		O ¹ Hal	O ³ III d U ³	O ⁴ t	2+3	-1?	d ^B III
33	M		Father of 31 and 34	R	WO'		O ³ II c	U ³	O ⁴ f U ⁴	2+3	-2	d ^{Co}
				L	(AC)W sm		O ³ Hal a	O ³ t	O ⁴ t	2+3	-2	
35	F		Sister B of 31 and 34	R	(AC)U'O't		O ² t	O ³ f	U ⁴			
				L	(AC)W ^{sma}		O ² t	O ³ t	O ⁴ t			d ^B
36	F		Sister M of 31 and 34	R	(AC)U'O't		O ² t	O ³ cl Bd U ³	O ⁴ t			d ^C
				L	(AC)U'O't		O ² t	O ³ cl BC	O ⁴ t			
37	F	thick webs between toes 2 and 3		<u>R</u>	(A)U'O't	Π^1 d	Π^1 d	U ³	O ⁴ cl Do	2+3		
				<u>L</u>	(A)U'O't		O ³ III d	U ³	O ⁴ t	2+3	-2	
38	M		Husband of 37	R	(AC)U'O't		O ² t	O ³ t	O ⁴ t			
				L	(AC)U'O't		O ² t	O ³ t	O ⁴ t			
39	M		Son H of 37 and 38	R	(A)U'O't		Π^1 f	Π^1 d	U ⁴	2+3		
				L	(A)U'O't		Π^1 c	U ³	U ⁴	2+3		d ^{CD}
40	M		Son B of 37 and 38	R	(A)U'O't		O ¹ III d	U ³	O ⁴ t	2+3	-1	
				L	(A)U'O't		O ¹ III d	U ³	O ⁴ t	2+3	-1	
44	M	skin bands between toes 2 and 3		<u>R</u>	(A)U'O't		O ¹ cl BC d	U ³	U ⁴	2+3	-1	d ^C
				<u>L</u>	W ^{sma} O't		O ¹ III d	O ³ III d U ³	O ⁴ t	2+3	-1	

TABLE 1—continued

No. and sex	Character of digital union	Relationship	Foot	Hallucal area	X	First interdigital area	Second interdigital area	Third interdigital area	Digital areas fused	Digital triradii absent	Proximal triradii
73 M	skin bands between toes 2 and 3		<u>R</u> <u>L</u>	Print (BC)U ^t O ^t	not	obtained O ^t	O ^t III d $\wedge$ III d	$\wedge$ d	2+3		d III
74 M	skin bands between toes 2 and 3		<u>R</u> <u>L</u>	(C)W ^a O ^t (C)W sm a		$\wedge$ t O ^{3t} $\wedge$ t	O ^{3t} O ^{3t}	O ^{4t} O ^{4t}	2+3 2+3		
93 F		Sister of 74	R L	(AC)U ^t O ^t (AC)U ^t O ^t		O ^{2t} O ^{2t}	O ^{3t} d BC O ^{3t}	O ^{4t} O ^{4t}			
75 M	skin bands between toes 2 and 3		<u>R</u> <u>L</u>	(AC)U ^t O ^t (AC)U ^t O ^t		O ^{3t} O ^{3t}	O ^{3t} O ^{3t}	O ^{4t} O ^{4t}	2+3 2+3	-2 $\wedge$ 2	
101 M	skin bands between toes 2 and 3	Brother of 75	<u>R</u> <u>L</u>	(C)W ^a O ^t (C)W sm a ^t		O ^{3t} O ^{3t}	O ^{3t} O ^{3t}	O ^{4t} O ^{4t}	2+3 2+3	-2 -2	
161 F		Sister of 75 and 101	R L	(C)W ^a O ^t (AC)U ^t O ^t		O ^{2t} O ^{2t}	O ^{3t} O ^{3t}	O ^{4t} O ^{4t}			
177 M		Brother of 75 and 101	R L	(AC)U ^t O ^t (AC)U ^t O ^t		O ^{2t} O ^{2t}	O ^{3t} O ^{3t}	O ^{4t} O ^{4t}			
178 M		Brother of 75 and 101	R L	(C)W ^t O ^t (C)W sm O ^t		O ^{2t} O ^{2t}	O ^{3t} O ^{3t}	O ^{4t} O ^{4t}			
77 (?W) F	skin bands between toes 2 and 3		<u>R</u> <u>L</u>	U ^t O ^t ? (BC)U ^t O ^t		O ¹ III d $\wedge$ t	W $\wedge$ t	O ^{4t} O ^{4t}	2+3 2+3	-1	
78 713W F	skin bands between toes 2 and 3		<u>R</u> <u>L</u>	W ^a O ^t (AC)U ^t O ^t		O ¹ III c U ^t O ^{3t} U ³ O ^{3t}	O ^{3t} O ^{3t}	O ^{4t} ? O ^{4t}	2+3 2+3	-1 -1? -2	d 1

202 M	Skin bands between toes 2 and 3; web between 3 and 4		R	(B)U ^t O ^t (BC)O ¹ II ^{III} d		O ¹ III ^d O ¹ II ^{III} U ¹ U ¹	∩ ^a O ⁴	O ^{4t} O ^{4t}	2+3+4 2+3+4	-1 -1, -3	
203 159W F	"slight syndactylism" (Wilder)		R	(AC)U ¹ O ^{1t} (AC)U ¹ O ^{1t}		O ^{3t} O ^{3t}	O ^{3t} O ^{3t}	O ^{4t} O ^{4t}	2+3 2+3	-2 -2	
204 156W F		Sister of 203	R	(AC)U ¹ O ^{1t}		O ^{3eld} ∩ ^a II ^c	U ³	O ^{4t}	2+3		d ^I III
205 158W M		Son of 204	L	(AC)U ¹ O ^{1t}		∩ ^t	O ^{3eld} B ³ III ^d U ³	O ^{4t}	2+3		d ^I III
206 157W M		Son of 204	R	(AC)U ¹ O ^{1t}		O ^{3t}	∩ ^t	O ^{4t}	2+3	-2	
207 397W M		Brother of 203	L	(AC)U ¹ O ^{1t}		O ^{3t}	∩ ^t	O ^{4t}	2+3	-2	
208 F	"marked syndactylism" (Wilder).		R	(AC)U ¹ O ^{1t}		O ^{1t} O ^{1c}	U ³	O ^{4t}	2+3	-1	d ^I III
210 M			L	(AC)U ¹ O ^{1t}		O ^{1t} (or ∩ ^t ?)	O ^{3t}	O ^{4t}	2+3		
211 84W F		Daughter of 210	R	(AC)W ^a O ^{1t}		O ^{1t} O ^{1III} d	U ³	O ^{4t}	2+3	-1	d ^I III
212 138W M		Grandson of 210	L	(C)W ¹ O ^{1t}		∩ ^t O ^{1t}	O ^{3t} U ³	O ^{4t}	2+3		d ^C II
213 85W F		Daughter of 210	R	(C)W ¹ O ^{1t}		∩ ^t O ^{1t}	O ^{3t}	O ^{4t}	2+3		
214 85W F		Daughter of 210	R	(C)W ¹ O ^{1t}		O ^{1III} d.O ¹ II	W	U ⁴ O ^{4t}	2+3+4	-1	
215 85W F		Daughter of 210	L	(C)W ¹ O ^{1t}		O ^{1III} d	WO ^{III} d	O ⁴	2+3+4	-1	
216 138W M		Grandson of 210	R	(A)U ¹ O ^{1t}		O ¹ II ^a	U ¹	∩ ^t	2+3+4+5	λ ¹ λ ² λ ³	
217 138W M		Grandson of 210	L	(AC)U ¹ O ^{1t}		O ^{1t}	U ¹³ O ^{3t}	O ^{4t} ∩ ^t	2+3+4+5	λ ¹ , -2	d ^I II

TABLE 1—Continued

No. and sex	Character of digital union	Relationship	Foot	Hallucal area	X	First interdigital area	Second interdigital area	Third interdigital area	Digital areas fused	Digital triradii absent	Proximal triradii
213 139 W F		Grand-daughter of 210	R L	(A)U'O't (C)WO't		$\cap^{II}$ $\cap^t$	W $O^t W O^{4t}$	$O^{4t} \cap^{cl}$ $\cap^t$	2+3+4 2+3+4		d II
245 F	skin bands between toes 2 and 3		<u>R</u> <u>L</u>	$W^t O^t$ $W^t O^t$	$\begin{smallmatrix} I \\ III \end{smallmatrix} d$ $\begin{smallmatrix} I \\ III \end{smallmatrix} a$	$\cap^{IIa} O^{III}$ $\cap^{III} d$	W W^a	O^{4t} O^4	2+3+4 2+3+4		
246 M	thin webs between toes 2 and 3		<u>R</u> <u>L</u>	(A)U'O't (C)W ^a O't	if	O^{2f} $O^{2t} O^{2f}$	$O^{3III} d U^3$ $O^{3f} O^{3III} d U^3$	$U^4 O^{4cl} B D$ $O^{4f} U^4$			d B III d ^I d ^{III}

Interdigital patterns

The figures and tables 1, 2, and 3 demonstrate the general variations of interdigital patterns which characterize syndactylous feet. Extreme deviations of the open fields and loops of the first

TABLE 2

Showing the distribution of pattern types of the first interdigital area; material from table 1, adapted to emphasize pattern type and primary relations to the interdigital spaces; R and L indicate right and left.

Ω	ΩO^1	U^1	O^1	O^2	O^3	$O^3\Omega$	$O^3U^3O^3$
17 L	34 R	202 L	13 R	246 R	20 R	74 L	78 L
37 R	245 R		13 L	246 L	20 L		
74 R			17 R		37 L		
77 L			26 R		75 R		
245 L			26 L		78 R		
			27 L		101 R		
			31 R		203 R		
			31 L		203 L		
			49 R				
			49 L				
			73 L				
			77 R				
			202 R				
			208 L				

TABLE 3

Showing the distribution of pattern types of the second interdigital area; material from table 1, adapted to emphasize pattern type and primary relations to the interdigital spaces; R and L indicate right and left.

W	Ω	U^1	O^1U^1	$O^3\Omega O^3$	$O^3O^3U^3$	O^3U^3	U^3	O^3	O^4
20 R	13 L	17 R	26 L	13 R	246 L	31 L	31 R	27 L	202 L
20 L	73 L	17 L				49 L	34 R	74 R	
77 R	77 L	26 R				246 R	37 R	74 L	
245 R	202 R						37 L	75 R	
245 R							49 R	78 R	
								78 L	
								101 R	
								203 R	
								203 L	
								208 L	

and second interdigital areas occur. Openings of the first interdigital area are diverted from their usual relation to the second interdigital space to the first or third spaces. Absence or disintegration of the first or second digital triradii is associated with the deviation. In the same manner, the second interdigital pattern is subject to deviation from its most common relation to the third interdigital space, its ridges being diverted frequently to the first or fourth spaces, correlated again with the absence or disintegration of critical triradii. The number of instances in which this pattern departs from its usual relation is much smaller than for the first; the second interdigital area is represented more frequently by whorls and inverted loops. Attention is called to the unilateral syndactylism of 27, 34, 75, and 101. In 34 only the syndactylous foot presents significant epidermal variants, while in each of the other cases both feet show similar variations. Neither the hallucal nor the third interdigital patterns presents variants of significance. As is true of fused digital areas, the variants of the first and second interdigital patterns are not confined to syndactyls. It is, therefore, the repetition in syndactyls which establishes a correlation between the configurations and digital fusion. The deviations and loss or disintegration of digital triradii are not correlated with thickness or extent or skin unions, since the variants occur even in the absence of such unions and without any correspondence to the degree of union when it is present.

DISCUSSION

Relation to the normal

Since skin bands and the thicker webs always are associated with fused digital areas, the latter are interpreted as a direct correlation with the digital condition. The converse association does not hold true; that is, not all individuals possessing fused digital areas have thick webs or skin bands. To determine the latter point, attention was directed to the digital area region of about 250 individuals who had only the usual thin interdigital membranes. Fused digital areas occur in approximately half the number.

What is the explanation of the fused digital areas of non-syndactyls? They are, conceivably, the proximal and abortive expression of the same tendencies which would produce actual syndactylism if extended distally. If this be true, they are either retained from an ancestral syndactylism or acquired ontogenetically through the introduction of a familial syndactylism which fails to express itself distally. Evidence furnished by inheritance indicates that at least some examples of fused areas in 'non-syndactyls' point to a rudimentary or abortive syndactylism. The causal factors in other cases may not be at all concerned with syndactylism, but with an independent involution of the plantar contours.

It would be of interest to compare the configurations existing in human syndactyls with those of other Primates which are normally syndactylous. The configurations of a large number of Primates are illustrated in the literature, particularly in the publications of Miss Whipple, Schlaginhaufen, and Kidd. Certain of these species are characterized by prominent webs or skin bands, but there is apparently no consistent correspondence between ridge directions at the bases of the digits and the type of union. In *Hylobates syndactylus*, for example, a species in which the second and third toes are united by a thick and extensive band, there is wide individual variation in the courses of ridges not only in the digital area region, but also upon the fused digits themselves (Schlaginhaufen, figs. 139 to 148, 152 to 154). The situation appears to be complicated by sources of variation which either do not exist in man or are present in less marked degree. If the conclusion that ridge direction is determined by embryonic growth stresses is a sound one, this view being discussed below, the sources of variation must reside in the far more prominent relief of the foetal *Hylobates* foot, as compared with the foetal human foot.

An association of aberrant first and second interdigital patterns with syndactylism has been established. It follows that they may be correlated causally, as the facts of inheritance indicate. Miss Whipple explains similar variations on the basis of degeneration or coalescence of the interdigital pads; thus, the signifi-

cance of variations centered chiefly in the first interdigital pattern is clear, since this is the zone of digital fusion. The similar variations in non-syndactyls are in the same status as fused digital areas, in so far as they may represent a proximal expression of syndactylism; otherwise they are to be explained by independent irregularities in the involution of the pads. There is, however, even here the possibility of an indirect relation between syndactylism and the irregularities of the pads. It is suggested that the zone of the second and third digits is more plastic and unstable owing to the digital modification in progress; hence this zone is particularly subject to developmental variations of the pads and, consequently, of the related patterns.

Three observations concerning triradii serve to illustrate the purely secondary rôle of these epidermal elements: 1) The accessory triradius related to the apical patterns of 202 has no homologue, and is merely the result of the apposition of two patterns. 2) The occurrence of triradii on skin bands and webs, as well as in fused digital areas, depends upon the fortuitous interrelationships of ridges, whose courses, in turn, are susceptible to variation through factors introduced by syndactylism. 3) Conversely, digital triradii suffer disintegration or loss through the secondary deviation of ridges of interdigital patterns. Triradii, then, are not morphological entities, but owe their existence to the chance interrelationships of ridges.

Inheritance

Evidence which supports the view that the epidermal variants, which have been described, are stigmata of syndactylism is furnished by several families in which one or more cases of syndactylism occur. At the outset, the inheritance of epidermal patterns may be outlined. Galton ('92) and Wilder ('04, '16, '19) present facts which show an inheritance of the general type of pattern; also Wilder ('16) traces a familial transmission of certain unusual patterns, such as the calcar. Miss Elderton ('20) has applied biometric methods in the study of heritability, confining the study to apical patterns of the fingers. She finds a parental-filial coefficient of correlation somewhat lower than

the coefficients for stature, span, and forearm. According to Wilder ('19), "Heredity determines with considerable precision the occurrence and arrangement of patterns and other larger features, but the execution of them, as drawn by the ridges, is wholly individual, and is quite beyond the limit of hereditary control." He asserts, further, in reference to identical twins, "... the correspondences are of those parts that are under the control of the germ plasm, and that where there is no definite correspondence the development is left to other forces, whatever they may be."

In the syndactyl material an unique situation exists. Both syndactylism and general pattern types are heritable, and definite pattern variants are found to be associated with syndactylism. At once is presented the question of whether the association is dependent upon a germinal correlation or a direct causal relation between syndactylism and epidermal variants. An analysis of the data which pertain to this question follows.

Both the mother and father (32 and 33) of syndactyls 31 and 34 (figs. 6 to 17) show evidence of the epidermal variations which characterize syndactylism, yet neither parent has skin bands or thick webs. Two sisters (35 and 36) of 31 and 34 show neither digital union nor critical epidermal variations. The origin of the skin bands in the syndactyls of this family is uncertain, since the known history is restricted to the above-mentioned members. It appears as if the skin-band condition were latent in the parent source, the syndactylous tendency being expressed, however, in the form of epidermal variants as superficial evidences of its proximal effects. After analyzing several extensive family histories of syndactylism, Schultz observes: "It can be stated, first of all, that apparently in no zygodactyl family does the anomaly skip a generation; i.e., those individuals who are free of the condition, although of zygodactyl strain, will in all probability have only normal children." This family is not an exception to the above generalization, as it would appear to be if only the skin-band condition were considered in rating syndactylism, but it illustrates an instability of inheritance of the degree of syndactylism. It will be recalled that 31 shows skin bands

bilaterally, while only the right foot of 34 is syndactylous—a circumstance which again illustrates this instability.

Although the mother (29) exhibits epidermal stigmata on one foot, neither parent of subjects 26 and 27 possesses webs or skin bands. The father (30) of these two syndactyls shows no critical epidermal variations, nor does their sister (28), whose patterns closely resemble those of the father (figs. 18 to 27). The patterns of 26 are strikingly different from those of either parent; in fact, they represent a radical departure from the usual pattern types. It is known that the second and third toes of the mother's sister are united by skin bands, which suggests a maternal source of the skin bands in 26 and 27.

An opposite case is presented by 37 (figs. 28 to 35), whose second and third toes are bound together by a thick and rigid web. Her husband (38) shows neither digital union nor irregularities of configuration, while both of their children (39 and 40) show epidermal variants without digital union. Both feet of one child (39) possess the general epidermal features of the mother's right one. The other child (40) has patterns on both feet which are similar to those upon the left foot of the mother, with the following difference: instead of a deviation of the first interdigital pattern to the third interdigital space, with loss of the second digital triradius, as in the left foot of the mother, the deviation is toward the first space and it is the first digital tri-radius which is lacking.

Another family, in which one member (203) shows slight syndactylism, demonstrates a strong inheritance of fused digital areas and interdigital pattern variants (table 1).

In one family (210 to 213, table 1) where three generations are represented there are sporadic traces of syndactylism, but no marked cases. The transmission of fused digital areas in this family is believed to be of significance with relation to the syndactylous tendency. The first generation represented is a man with fused digital areas on both feet (210; his right foot is illustrated in Wilder and Wentworth, fig. 60) and his wife, who lacks such fusion. Their two daughters (211 and 214) have fused digital areas bilaterally. Prints of a son are not available. Two

children of the son (212 and 213), whose wife also is not available, have fused digital areas. In one of them (212; illustrated in Whipple, pl. V, a and b) the fusion is so extensive as to include all four areas. One daughter has no children. The remaining daughter has three, and only one of these shows fused digital areas; this one, moreover, has a decided suggestion of syndactylism. Their father has fused digital areas on one foot only.

Since it is true that the filial generations may be truly syndactylous, even though their parents show only critical epidermal variants, and that the converse relation holds also, the epidermal variants are assumed to be stigmata of syndactylism even in the absence of digital union. Inasmuch as cases are presented in which the stigmata are not alike in parent and offspring, it appears that the introduction or the exaggeration of syndactylous factors already present is capable of modifying the heritable factors which control configurations. It follows that the variants are correlated with syndactylism, not through the germ plasm, but through factors introduced by the anomaly.

Relation to the genesis of pattern forms

Theories which have been advanced to account for the directions of epidermal ridges depend upon the functions which are attributed to them; therefore, the more recent views concerning their functions are outlined briefly. Galton states, "The uses of the ridges are primarily, as I suppose, to raise the mouths of the ducts so that the secretions which they pour out may the more easily be got rid of; and secondarily, in some obscure way to assist the sense of touch." Hepburn ('95) asserts that the ridges serve both as useful friction points and as a means of increasing the tactile sensitivity of the epidermis. Féré ('95; '96) believes the ridges to be adaptations to the tactile function. Miss Whipple concludes, "That the function of the ridges is primarily to increase resistance between contact surfaces for the purpose of preventing slipping, whether in walking or prehension." Wilder's publications carry the same idea of function. Both Schlaginhaufen and Kidd ('05, '07, '09) emphasize the utility of ridges as a means of increasing the sensitivity of the

palmar and plantar surfaces. Kidd summarizes the problem of function:

There are two main views of the functions of the papillary ridges which cover the ventral surface of the hand and foot of many mammals. One is that they have a mechanical function of preventing slipping in the acts of prehension and walking, and the other is that their function is primarily tactile and that they subserve the sense of touch. The former view does not exclude the latter, nor the latter the former; the controversy between those who hold one or the other of these views affects only the question of the arrangement of these specialized structures of the skin, and the primacy of one over the other.

The courses of ridges and the often complex configurations which they form are explained variously by different writers. Kollman ('83; '85) accounts for ridge direction on the basis of growth stresses. Hepburn, while discounting Kollman's view, holds, "the production of any special 'design' or 'pattern' is to some extent an accidental occurrence." He regards the pattern variations as resultant from variations in the shapes of the eminences with which they are related. Féré, Schlaginhaufen, and Kidd all regard the ridge arrangements primarily as means of increasing the tactile usefulness of the epidermis. Miss Whipple and Wilder hold to the view which is summarized by the former as follows: "The direction of ridges is at right angles with the force that tends to produce slipping, or to the resultant of such forces when these forces vary in direction. The shape of the pad elevation, the direction of flexion, and the direction of motion, are the factors determining the direction of the slipping force, and therefore the direction of the ridges."

We have shown that ridge direction is subject to modification in syndactylism. This modification cannot be explained by the postulation of a germinal correlation between syndactylism and the epidermal variants as such; nor is it reasonable to assume that the modification is an abrupt adaptation to new tactile or friction needs of the individual. While the syndactyl variants are far more aberrant than those variations referred by Wilder ('19) to the control of non-germinal factors, it appears that both may result from the action of like factors, the identity of which can be ascertained in the case of syndactylism.

It is obvious that new stresses in embryonic growth are operating within fused digits. In the immediate digital region these stresses bring about fusion of the digital areas and continuity of the basal transverse ridges. New stresses probably are not confined to the digits and their immediate neighborhood. Both Weidenreich and Schultz note cases of cutaneous syndactylism in which the proximal effects of syndactylism are evident in extensor tendons, and Miss Hays ('17) describes a high-grade syndactylous cat in which the walking pads, as well as internal structures of the feet, are abnormal. The interdigital pads in man, then, might, likely enough, undergo coalescence or other modification as the result of syndactylism, varying the pattern forms through the changed stresses thus induced. The juxtaposition of two apical pads, in 202, introduces a totally different growth stress complex than would occur if the two pads were not apposed, so accounting for the novel ridge directions in their vicinity and the consequently aberrant triradii. A localization of the effective growth stresses within the mesoderm is suggested by the definite correspondence of ridge direction and thickness of skin union; that is, transverse ridges are characteristic of all skin bands and the thicker webs, while the ridges are longitudinal in cases of thin webs, whether in syndactyls (246) or 'non-syndactyls.'

In addition to the foregoing conclusion derived from the syndactyl material only, the writers have been led to the tentative view that, whatever may be the factors which initiate ridge formation, the direction of ridges in general is determined by ontogenetic growth stresses. This idea does not conflict with the prevailing views concerning the functions of epidermal ridges, nor with the facilitation of function mediated through their arrangement in patterns. There is, however, an obvious disharmony between this explanation and that of use, as advanced by Miss Whipple, Kidd, and Wilder, to account for pattern forms. The explanations which are centered about use assign to the relief of the hand or foot only a secondary rôle in the control of ridge direction. The ultimate factors which are assumed to control ridge direction are extrinsic, and the irregu-

larities of contour serve merely to modify the action of these extrinsic factors. But according to the view which delegates the control of ridge direction to growth stresses, the determining factors are intrinsic, varying lines of stress induced within the organism itself.

No other explanation seems to account for the wide range of variation occurring in man, or in any Primate species having well-defined ridges. It offers an explanation for the variations in identical twins and for the looseness of inheritance of patterns, in full accord with the indication that the vehicle of inheritance is not infallible, that it is a loosely governed and labile mechanism. It clears up the question concerning the variations which sometimes are found in equivalent digits in hyperdactylism. Also, the usual association of recurved or concentric ridges with higher eminences and of more or less straight and parallel ridges with flat areas or elongated parts, such as the proximal segments of digits and the prehensile tails of certain Primates, suggest the responsibility of growth stresses as determiners of ridge direction. The controversial issue raised by Kidd ('07) concerning the history of patterns upon secondary pads loses force if growth stresses induced within these pads are responsible for their patterns. The phylogenetic history of primary pattern forms parallels the history of the pads, the pads being vortices of growth stresses which are more complex than those which occur in relation to flat surfaces.

A significant relation, which has not received due attention, exists with reference to the embryonic period in which the ridges are formed and the contours of the palmar and plantar surfaces at that time. It may be unwise to compare the complexity of pattern forms with heights of pads in postnatal individuals only; however, the association of complex patterns with higher eminences is evident in general. Pads are prominent in the early human foetus (Johnson, '99; Retzius, '04). As development progresses they become relatively less and less prominent despite which the already formed patterns remain unchanged. It is this which may explain Miss Whipple's observation, ". . . that so strong is the tendency of the ridges to follow the relief

of the pads that even pads which have lost their typical elevated form occasionally show as a variant the primitive typical concentric arrangement of ridges with the full quota of triradii upon a practically flat surface, indicating that long after pad degeneration has been accomplished, the typical ridge arrangement may persist or recur to tell the story of a former condition." Miss Whipple refers to the 'former condition' in phylogeny, not in ontogeny.

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EXPLANATION OF FIGURES

Each figure of sole patterns is drawn as if from the sole itself, thus correcting the transposition which occurs in contact prints. The outlines of the larger figures are tracings representing the actual outlines of the feet, while the outlines of the smaller figures are diagrammatic. Syndactyls are distinguished by the fact that their accession numbers are in bold-faced type.

PLATE 1

- 1 Right, **202**.
- 2 Left, **202**.
- 3 Photograph of right foot, **202**.
- 4 Distal extremity of digital mass, right foot, **202**.
- 5 Tibial aspect of digital mass, right foot, **202**.

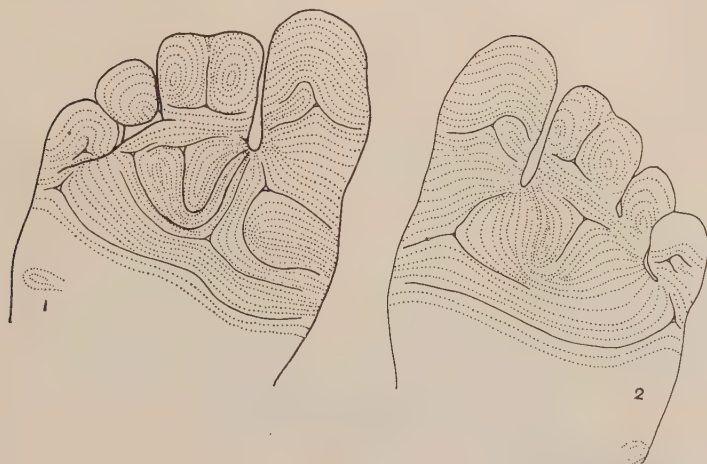
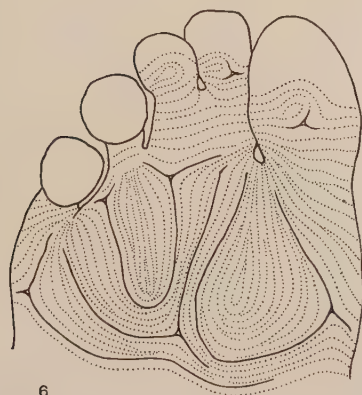


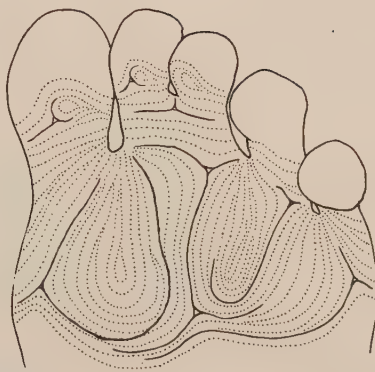
PLATE 2

EXPLANATION OF FIGURES

- 6 Right, **31**.
- 7 Left, **31**.
- 8 Right, 32, mother of **31**.
- 9 Left, 32.
- 10 Right, 33, father of **31**.
- 11 Left, 33.
- 12 Right, **34**, sister R of **31** (only right foot is syndactylous).
- 13 Left, **34**.
- 14 Right, 35, sister B of **31**.
- 15 Left, 35.
- 16 Right, 36, sister M of **31**.
- 17 Left, 36.



6



7



8



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12



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14



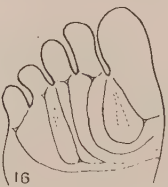
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17

PLATE 3

EXPLANATION OF FIGURES

- 18 Right, **26**.
- 19 Left, **26**.
- 20 Right, **27**, sister of **26**.
- 21 Left, **27**. (Only left foot is syndactylous.)
- 22 Right, 28, sister of **26**.
- 23 Left, 28.
- 24 Right, 29, mother of **26**.
- 25 Left, 29.
- 26 Right, 30, father of **26**.
- 27 Left, 30.

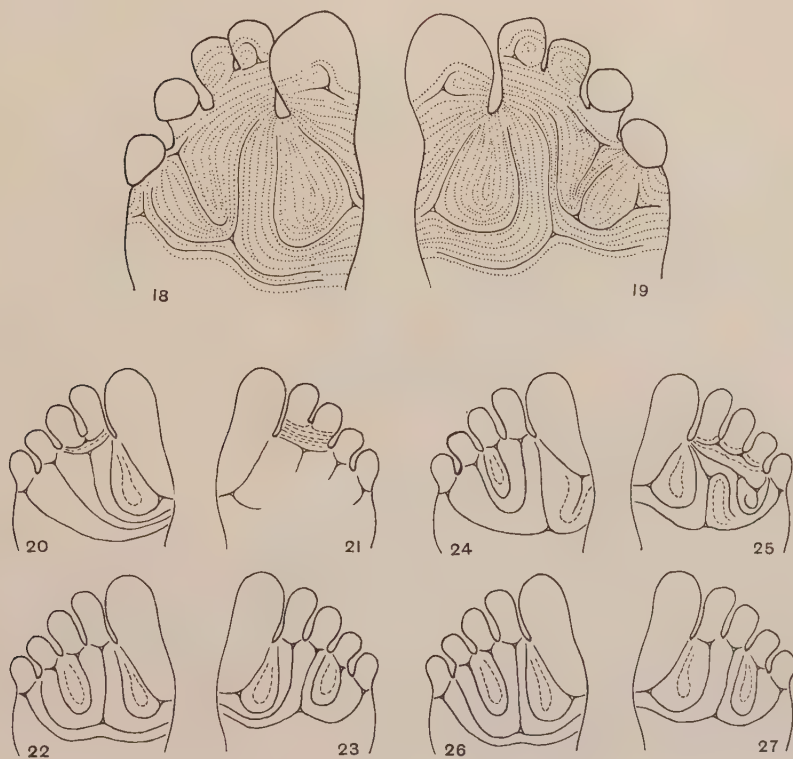
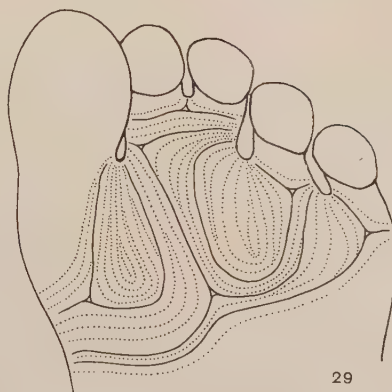
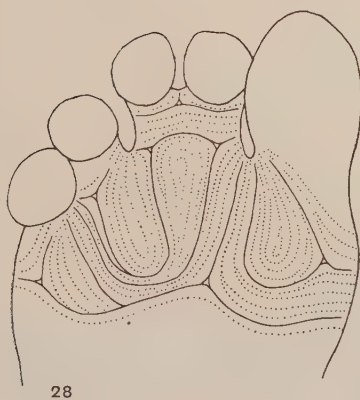


PLATE 4

EXPLANATION OF FIGURES

- 28 Right, **37**.
- 29 Left, **37**.
- 30 Right, 38, husband of **37**
- 31 Left, 38.
- 32 Right, 39, son H of **37** and 38.
- 33 Left, 39.
- 34 Right, 40, son B of **37** and 38.
- 35 Left, 40.



THE INNERVATION OF THE CAROTID BODY

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TWO FIGURES

INTRODUCTION

For many years investigators believed the carotid gland to be a ganglion of the cervical sympathetic trunk. This idea originated with Haller, who in 1743, first noticing a rounded body lying close to the common carotid artery in the region of its bifurcation, assumed it to be a sympathetic ganglion. Following Haller, there were several investigators who adhered to this assumption; Berkelmann in 1744; Newbauer, 1772; Andresch, 1797, who named it 'ganglion intercaroticum,' and Mayer in 1833. Mayer added to the previous data a description of a band of connective tissue uniting the 'ganglion' to the artery, the so-called ligament of Mayer. He also described and named a plexus of nerve fibers (*nervus mollibus*) in relation to the body, which plexus received fibers from the superior sympathetic ganglion, the glossopharyngeal, and the vagus nerves.

In 1833, Valentine found the so-called 'ligament' of Mayer to be a band of nerve fibers passing between the 'ganglion' and the artery. He saw that the 'ganglion' was completely surrounded with nerves, while branches of these nerves left it from both sides. Some of these branches passed to the artery and others joined the '*nervus mollibus*.' He also described a special branch from the vagus to the 'ganglion.'

Luschka ('62) gave a new impetus to the study of this structure when he offered the first microscopic description of it. He denied that it was a sympathetic ganglion as his sections showed a

¹ Contribution no. 101.

distinctly glandular structure. He also found that the gland was supplied with nerve fibers from the 'intercarotid plexus' which consisted of a network of fibers from the superior laryngeal and the glossopharyngeal nerves, and the superior cervical sympathetic ganglion. From this plexus fibers passed to the gland, interlacing with the glandular elements. He believed that the plexus also supplied fibers to the carotid artery and its branches. Luschka also described, besides the gland cells in the carotid body, other cells arranged in groups of three or four which he regarded as sympathetic ganglion cells. A few of these were multipolar, but most of them were unipolar.

In the year following Luschka's contribution, a very careful description of the gland was published by Switzer. He confirmed Luschka's description of the innervation of the gland, and described in addition fibers from the hypoglossal nerve to the 'intercarotid plexus.'

Arnold, even three years later, called the gland a ganglion of the sympathetic trunk, and pictured the 'intercarotid plexus' as receiving fibers only from the glossopharyngeus, the vagus and the superior cervical sympathetic ganglion. There were more unmyelinated than myelinated fibers in the gland and the ganglion cells were mostly unipolar. Marchand, writing in 1891, merely mentioned the presence of many nerves about the gland, and Schaper, a year later, found in a gland stained by Pal's method a heavy network of nerve fibers. What is more significant, Schaper found far fewer ganglion cells than had previously been described, and those which he did find were multipolar. In 1895, Kopfstein mentioned fibers to the gland only from the superior cervical sympathetic ganglion; Princeteau ('99) found either three or four nerves connecting the superior pole of the gland and the intercarotid plexus, while Kohn ('00) found many nerve bundles which separated at the periphery of the gland into individual fibers which ended among the gland elements and among sympathetic ganglion cells as preganglionic fibers. Kohn believed that only the branch from the superior sympathetic ganglion to the gland was constantly present.

More recent work of Funke ('04), Gomez ('08), and Bethe

('15) had not contributed any further information about the innervation of the gland, though a very complete review of the literature on the carotid body in all its aspects may be found in Funke's article.

TECHNIQUE

For the present investigation material was examined from seven cats, five dogs, two full-term human fetuses, and two adult human bodies. Two types of dissections were made. In the case of the human material, the area at the region of the bifurcation of the carotid artery was first exposed, the gland located, and the connective tissue carefully teased from the vicinity of the gland. This procedure exposed the nerves as they passed into the gland, from which they could be followed to their several origins, and left intact the communications which, on gross examination, resembled plexuses. A similar method was employed in the dissection of two of the cats and two of the dogs. In the other cases the cervical sympathetic trunk and the glossopharyngeal, vagus, and hypoglossal nerves were first exposed in the region of the gland, and any branches of these going in the direction of the bifurcation of the carotid artery were carefully followed and their relations to the carotid gland determined.

When a clean dissection had been made, a cover-glass was slipped under the gland and artery, and the various nerves freed from the main nerve trunks and tied around the glass in such a manner as to preserve their original relations. A cut was then made in the common carotid artery at 3 to 5 mm. below the bifurcation, and a cut in the internal and external carotid arteries the same distance above the bifurcation. For reference in the histological examination, a careful record of the gross relations was kept. In one dog the gland and its nerves were freed from the artery also, tied on a cover-slip and imbedded in spinal cord before fixing (for method see Ranson, '18). Material from two cats, one dog, and one man were not fixed, the dissections being made for gross relations only. That from three cats, one dog, and one man was fixed in Zenker's fluid and stained with haematoxylin and eosin. From two dogs and two cats the material was fixed in ammoniated alcohol, and stained by the pyridine-

silver technique (Ranson, '11). All of the material was imbedded in paraffin, cut and mounted in serial sections, 8 to 10 μ thick.

GROSS ANATOMY

In cats the variations in the gross findings were very slight. The carotid gland consisted of a small oval reddish body varying from 1 to $\frac{3}{4}$ mm. in diameter, and situated in the connective tissue behind the bifurcation of the carotid artery, so that the dissection was made more easily from the deep surface of the vessel. A small branch of the glossopharyngeus, passed to the upper pole where it broke into many small filaments which entered the gland substance, and of which in one cat one continued beyond the gland and ascended the external carotid artery in company with the nerves from the superior cervical sympathetic ganglion to the external carotid plexus. Another nerve arose near the middle of the superior cervical sympathetic ganglion and passed medially and inferiorly to reach the bifurcation of the carotid artery, where a part of it entered the gland; the rest of it continuing to the external carotid artery, upon the medial part of which it ascended. In all the cats examined no fibers other than those from the sympathetic ganglion and glossopharyngeal nerve were seen to enter the carotid gland, nor was there any indication of an 'intercarotid plexus' between the internal and external carotid arteries.

The structures in the dog were much more variable than in the cat. In the region of the posterior surface of the common carotid artery there was a very small flattened reddish body which was closely adherent to the wall of the blood vessel. Other small patches of this reddish material could be seen with the naked eye, scattered on the wall of the common carotid artery below the bifurcation and, a few, on the wall of the external carotid. None of this material was seen more than a centimeter away from the bifurcation, nor did it appear anywhere upon the internal carotid. In every case (except that of one dog) there was a plexus of nerve fibers between the internal and external carotid arteries, from 3 to 7 mm. from the angle of the bifurcation, which contributed fibers to the main carotid gland and to the

external carotid artery. Fibers entered this plexus from the inferior pharyngeal branch of the glossopharyngeus, from the middle part of the superior cervical sympathetic ganglion, and in two dogs from the vagosympathetic trunk. In one dog the last fibers were carefully followed centrally and were seen to plunge into the superior cervical sympathetic ganglion about a centimeter above the region where the fibers left the trunk. In one dog an additional nerve from the same ganglion passed directly to the main carotid gland without joining the plexus, while in another dog, where no plexus was evident, the branch from the glossopharyngeus passed directly into the gland, and the branch from the sympathetic reached the gland from the external carotid nerve.

The two fetuses examined were identical twins and, as might be expected, the findings were similar. The gland was a small oval mass of reddish material, situated at the bifurcation of the common carotid artery. A branch of the inferior pharyngeal ramus of the glossopharyngeus passed directly into the upper pole of the gland, where just before entering it gave off a few fibers to the pharyngeal plexus. There was also a nerve to the gland from the superior cervical sympathetic ganglion, most of the fibers of which pierced the gland, while a number continued beyond to ascend the external carotid artery.

The findings in the two adult men examined were similar to those described for the fetuses, except that the gland was flatter, less regular in outline, and more closely applied to the wall of the artery. There was no evidence of an intercarotid plexus, nor of branches from the vagus or hypoglossus to the gland.

HISTOLOGICAL DESCRIPTION OF THE INNERVATION

A. From the central nervous system

The branch from the glossopharyngeus was quite similar in the different animals studied. The nerve separated from the main trunk in the form of a single fiber bundle from 320 μ to 360 μ in diameter. (In the dog it was usually of the smaller dimension.) The majority of the fibers belonged to the small

myelinated class; the largest, varying from 6 to 12 in number and from 9 to 12 μ in diameter, were scattered irregularly throughout the fascicle in groups of two or three. Next in size were those of 5 to 8 μ in diameter. There were fifteen to twenty of these which, in several cases, were somewhat aggregated at one

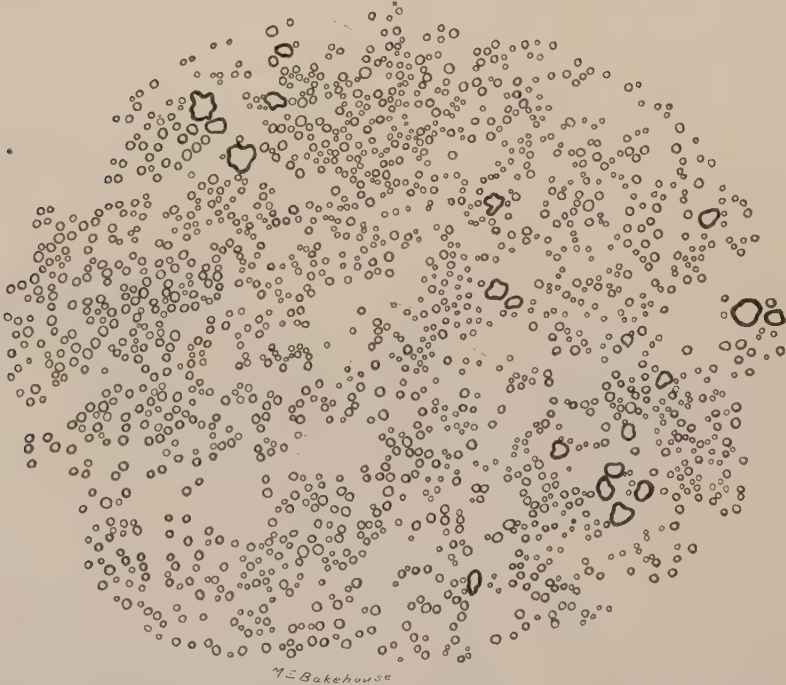


Fig. 1 Osmic acid. A cross-section of the branch from the glossopharyngeal nerve to the carotid gland in the cat. $\times 453$.

side of the nerve bundle. The smallest fibers of 2 μ to 4 μ were very numerous and appeared in clusters, which, in osmic-acid preparations, were separated by rather large clear spaces. These spaces were more noticeable near the center of the nerve than at the periphery (fig. 1). The total fiber content was similar in all the animals studied, but was constantly greater in the cat than in the dog, whereas in the dog the number of large fibers was greater than in the cat.

The pyridine-silver preparations showed a similar arrangement of large and small myelinated fibers. In addition, however, there were unmyelinated fibers dispersed irregularly between the patches of myelinated fibers, in groups of four or more. In one dog the nerve just described received an additional bundle of unmyelinated fibers from the branch of the sympathetic to the external carotid artery which joined this nerve a short distance before it entered the gland.

Very soon after leaving the main stem of the glossopharyngeus, the nerve to the gland divided into two or three bundles, in which the fiber relations remained the same as within the undivided nerve, like fibers showing no tendency to be grouped in a single bundle. In the region of the gland further subdivision into twenty-four to thirty-two bundles occurred. These varied in size from large ones of 72 μ in diameter to small ones with no more than two small fibers in them. The larger bundles remained at the periphery of the entering nerve, the smaller and more numerous bundles at the center. Within the gland, the bundles became separated by the cell masses, gradually breaking into smaller groups, some of which passed directly through the gland, and into single isolated fibers, all of which terminated in it. These individual fibers left the bundles shortly before terminating between the individual cells or cell groups.

As a rule, the myelin sheaths were lost by the time the fibers started their independent courses. Several of the sections from osmic-acid preparations showed isolated fibers with myelin sheaths, which, however, terminated rather abruptly between the cell groups. In the pyridine-silver preparations the fibers could be traced beyond the myelin when the axis cylinder stained more deeply and lost the clear ring immediately surrounding it. The final termination of all the fibers traced was at the periphery of the individual cells (fig. 2).

Those groups of fibers which did not terminate within the main mass of the carotid gland passed through it and joined one of the fiber bundles which had remained at the periphery. These combined bundles ascended the external carotid artery, sometimes alone and sometimes in company with the nerve from the

superior cervical sympathetic ganglion to the external carotid plexus. The bundle of fibers from the branch of the glossopharyngeus so distributed was particularly large in the dog. When these fibers were followed upward, they were seen to end in the masses of carotid gland tissue which were scattered along the external carotid artery.



Fig. 2 Pyridine silver. A group of cells in the carotid gland, showing the relation of the nerve fibers to the cells. $\times 1813$.

B. From the sympathetic nervous system

The fibers which passed to the gland from the superior cervical sympathetic ganglion were similar to the postganglionic fibers described by Ranson ('18). The fascicle before dividing measured in general 170 to 220 μ in diameter, and consisted mainly of unmyelinated fibers, though eight to twelve myelinated fibers were usually found. Upon reaching the bifurcation of the carotid artery, some of the fibers entered the gland, but the majority of them, in the dog, ascended the external carotid artery in company with the nerve from the superior cervical sympathetic ganglion to the external carotid plexus, and in some cases could be followed into the glandular masses along the artery. In the cat a larger number of fibers entered the main carotid body than in the dog. The final termination of all the bundles could not be ascertained, but those which did not enter carotid gland tissue

continued upward in the direction of the external carotid plexus, while those which did enter the gland separated very soon into smaller bundles or into individual fibers which wove their way through the cell groups. The final termination of these fibers could not be determined.

NERVE CELLS

In the search for sympathetic nerve cells, we examined serial sections of the gland stained with haematoxylin and eosin and others stained with pyridine-silver. As far as we were able to determine, there were no nerve cells of any type within the gland. The cytoplasm of the gland cells stained lightly with a basic stain, and therefore resembled, to a slight extent, the cytoplasm of nerve cells. We are inclined to think that the few nerve cells which have been attributed to the gland in the past, were the larger of the chromaffin cells, for these are often irregular in outline and possess large, clear nuclei. This may be correlated with the observations of Elliott ('13), who was unable to find any nerve cells in the largest of the chromaffin organs, the suprarenal.

DISCUSSION

The relation of the gland cells of the carotid body to the entering nerve fibers is very similar to the relations found in a sympathetic ganglion. The fibers from the glossopharyngeus terminate in intimate relation to the individual gland cells and may be regarded as secretory. In the absence of intraglandular ganglion cells with which the glossopharyngeal fibers might form synapses constituting a two-neurone system, the gland cells themselves may be regarded as playing this rôle. The fibers from the glossopharyngeus may therefore be considered as cranial preganglionic fibers and the gland cells as occupying the position of ganglion cells. The derivation of the chromaffin cells from the sympathetic system is significant in this relation.

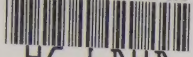
A similar relation in the adrenal has been suggested by Kohn ('00) and especially by Elliot ('13), who points out the similarity of ultimate function of the chromaffin and the ganglion cells. "The one affects directly by the nervous impulse and the other

by adrenalin excreted into the blood stream. Their present anatomical separation may be an index of a differentiation of functions which were once held by the two in common, when the adrenalin liberated was part of the nervous impulse and the 'paraganglion' cell was part of the ganglion cell." Our findings in the carotid gland are in accord with this view.

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